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Health and Environmental Effects Profile for Pentachloronitrobenzene

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16 ABSTRACT

The Health and Environmental Effects Profile for Pentachloronitrobenzene was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Pentachloronitrobenzene has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for pentachloronitrobenzene is 2.62×10^{-1} $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for pentachloronitrobenzene is 1000.

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR PENTACHLORONITROBENZENE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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The initial draft of this report was prepared by Syracuse Research Corporation under Contract No. 68-03-3228 for U.S. EPA's Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Dr. Christopher DeRosa was the Technical Project Monitor and Helen Ball was the Project Officer. The final documents in this series were prepared for the Office of Solid Waste, Washington, DC.

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EXECUTIVE SUMMARY

Pure pentachloronitrobenzene (PCNB) is a colorless crystalline solid (Worthing, 1983). The commercial product may have a light-yellow to cream color with a musty odor (Hartley and Kidd, 1983). It is practically insoluble in water but is soluble in a number of organic solvents. The compound is reasonably stable but may undergo hydrolysis in a strong alkaline medium (Hartley and Kidd, 1983). In 1983, Olin Corp., Leland, MS, was the only manufacturer of PCNB in the United States (SRI, 1984; Hartley and Kidd, 1983). No data for U.S. production volume for this chemical are available, but recent production source data (USITC, 1985; SRI, 1985) suggest that this chemical is no longer commercially produced in the United States. The primary usage of PCNB is as a soil fungicide for a wide variety of crops and in seed treatment (Worthing, 1983; Hartley and Kidd, 1983).

The fate of PCNB in water has not been comprehensively studied. Only qualitative data regarding fate and transport in water are available. The half-life of PCNB in the water phase was estimated to be 1.8 days. The two processes reported to be most responsible for the rapid decrease in PCNB concentration in water were volatilization and sorption to seston and biota, followed by sedimentation as detritus (Schauerte et al., 1982). Neither biodegradation nor photolysis appears to be a significant process for the loss of PCNB from water (Crosby and Hamadmad, 1971; Schauerte et al., 1982). The BCFs for PCNB in the golden orfe, Leucisens idus melanotus, and in rainbow trout, Salmo gairdneri, were reported to be 950-1130 and 260-590, respectively (Korte et al., 1978; Oliver and Niimi, 1985). It therefore appears that PCNB will moderately bioaccumulate in aquatic organisms.

Pertinent data regarding the fate and transport of PCNB in air could not be located in the available literature as cited in the Appendix. Based on its physical properties and its behavior in other media, it would appear that PCNB will persist in the atmosphere because no known chemical/photochemical processes significantly degrade this chemical. Precipitation of particulate PCNB, especially of larger particle size and higher particle density, may remove some PCNB from the atmosphere.

PCNB is persistent in soils. The two processes that are important in the loss of PCNB from soils are volatilization and biodegradation; biodegradation is more rapid in soils under anaerobic conditions than under aerobic conditions (Ko and Farley, 1969; Casley, 1968; Gile and Gillett, 1979; Cole and Metcalf, 1977). The average half-life of PCNB in a variety of field soils was reported to be 434 days (Beck and Hansen, 1974). Another study reported the half-lives for PCNB in sandy loam, clay and peaty muck soils as 4.7, 7.6 and 9.7 months, respectively (Wang and Broadbent, 1972). PCNB is expected to be moderately mobile in soils (Gile and Gillett, 1979). Evidence of very restricted (~3% on the average) translocation of PCNB and its metabolites from the roots to foliar tissue in plants was reported by Cairns et al. (1983).

Pertinent data regarding the levels of PCNB in ambient and drinking water could not be located in the available literature as cited in the Appendix. U.S. EPA's STORET database contains 120 entries on the analysis of PCNB in ambient water and aqueous effluent samples. Maximum and minimum PCNB concentrations in these samples were 1.0 and 0.01 µg/ℓ, respectively, with a mean value of 0.4 µg/ℓ.

Pertinent data regarding the ambient atmospheric level of PCNB could not be located in the available literature as cited in the Appendix. The concentration of PCNB in the ambient air of a commercial pest control building was reportedly 1.56 µg/m³ (Yeboah and Kilgore, 1984).

In the foods consumed by U.S. adults, infants and toddlers, PCNB residues have been detected mainly in the oils, fats and shortening group of foods (Johnson et al., 1981a,b, 1984a, Johnson and Maske, 1976; Maske and Johnson, 1975; Podrebarac, 1984a,b). Occasionally, PCNB has been detected in sugar and adjuncts, fruits and potatoes (Manske and Johnson, 1977; Johnson and Manske, 1977; Podrebarac, 1984a). On the basis of the assumption of a maximum PCNB content of 0.006 mg/kg (Johnson et al., 1984a) in oil, fats and shortening composite and a daily adult consumption of 0.070 kg/day of this category of food (Johnson et al., 1984a), the maximum daily adult intake of PCNB in the United States has been estimated to be 0.4 µg. The Canadian daily PCNB intake has been reported to vary between 0 and 0.4 µg (McLeod et al., 1980).

Little pertinent information is available concerning toxicity of PCNB to aquatic organisms in the available literature. PCNB toxicity to carp increased with increasing temperature, with 24-hour LC_{50} values ranging from 40 mg/ℓ at 15°C to 1 mg/ℓ at 35°C (Hasimoto and Nishiuchi, 1981). The lowest concentration at which toxic effects were reported for any species was 1 mg/ℓ. PCNB and its metabolites can apparently accumulate to a significant extent in fish and other aquatic biota. In the species that have been studied, PCNB metabolism involves denitrification but not dechlorination (Bahig et al., 1981; Murphy et al., 1982).

Pharmacokinetic and metabolism studies of PCNB indicate that it is rapidly and extensively absorbed from the gastrointestinal tract of rabbits, rats and monkeys (Betts et al., 1955; Korte et al., 1978; Koegel et al., 1979a,b). Following absorption by monkeys and rats, PCNB is rapidly metabolized and the metabolites are widely distributed to tissues but do not accumulate (Koegel et al., 1979a,b; Borzelleca et al., 1971). Courtney et al. (1976) demonstrated that PCNB and its metabolites can cross the placental barrier of mice. Major metabolites in urine and feces of monkeys and rats include pentachloroaniline, pentachlorothioanisole, pentachlorobenzene, pentachlorophenol, pentachlorothiophenol and mercapturic acid conjugates (Borzelleca et al., 1971; Koegel et al., 1979c). Metabolic pathways in monkeys involve reduction of the nitro group to pentachloroaniline and conjugation with glutathione. Conjugation twice with the same molecule of PCNB can lead to bis-methylmercapto-tetrachlorobenzene and other tetrachlorometabolites. Conjugation and nitro reduction combined can lead to tetrachloroaminometabolites. Similar pathways may operate in rats. The metabolites are excreted in the urine and bile. Biliary excretion accounts for the fecal metabolites. Unchanged PCNB was excreted only in the feces of monkeys. The rate of excretion of PCNB and metabolites was dose-dependent: 50% was excreted in 24 hours following a dose of 0.5 mg/kg; and 50% was excreted in 4 days following a dose of 91 mg/kg.

There have been five chronic bioassays involving dietary administration of PCNB. BRL (1968a) administered an average concentration of 1298 ppm PCNB to 18 male and 18 female B6C3F1 and B6AKF1 mice for 77 weeks. Courtney et al. (1976) later confirmed that the sample was contaminated with 11% hexachlorobenzene (HCB). The only positive neoplastic results were increased incidences of hepatomas in female B6C3F1 and male B6AKF1 mice

($p<0.001$), compared with negative controls. Cabral et al. (1977, 1978), however, noted increased incidences of this tumor type in hamsters treated with 50-200 ppm and mice treated with $\geq$100 ppm HCB alone in the diet.

NCI (1978) administered 97% PCNB to 50 male and 50 female Osborne-Mendel rats and B6C3F1 mice for 78 weeks, at two dietary levels. Average concentrations for the low-dose rats were 5417 ppm (males) and 7875 (females) ppm, and for the high-dose rats were 10,064 ppm (males) and 14,635 ppm (females). Average concentrations for the low-dose mice were 2606 ppm (males) and 4093 ppm (females), and for the high-dose mice were 5213 ppm (males) and 8187 ppm (females). Compared with control groups of 20 rats each, the treated rats showed no significant increases in tumor incidence. Among the mice, low-dose males and high-dose females did not show any significant (by the Fisher exact test) elevations in hepatocellular carcinomas, over control rats. These results were especially difficult to evaluate because of the small number of male mice that survived to terminal sacrifice and the large number of tissue sites that were autolyzed in female mice. In a repeat study by NTP (1986) that used highly purified PCNB (>99% PCNB, 0.07$\pm$0.01% HCB), B6C3F1 mice were given the compound in the diet at 2500 or 5000 ppm for 103 weeks. No evidence of carcinogenicity was found, although high mortality in the treated female mice decreased the sensitivity of the study.

Two oral chronic bioassays using technical grade PCNB were performed in Swiss mice (Van der Heijden and Til, 1974) and albino rats (Anonymous, n.d.). The compound was administered for 80 weeks (mice) and 104 weeks (rats), at 0, 100, 400 or 1200 ppm. In high-dose female mice there were increased ($p<0.001$) incidences of fibrosarcomas and combined fibromas/fibrosarcomas ($p<0.001$), relative to control values. By employing Cochran-Armitage trend tests statistically significant ($p<0.001$) differences were

observed in female mice for these tumor types. In male mice, there was suggestive evidence of a treatment-related increase in liver nodules, which was probably a hyperplastic rather than a neoplastic effect. In rats of either sex, there appeared to be no treatment-related increase in tumorigenesis.

An analysis of the possible influence of the technical grade contaminant HCB shows there to be a reasonably close numerical relationship between the HCB level and the estimated carcinogenic potencies of the technical grade PCNB compared with the HCB alone. A similar relationship seems to exist for the tumor response. In view of the available data, it is reasonable to observe that the technical grade contaminant, HCB, may be influential in the observed carcinogenic activity of PCNB. Using weight of evidence criteria from the U.S. EPA (1984) proposed guidelines, PCNB is considered to have limited evidence for human carcinogenicity; thus it can be classified as an EPA Group C compound.

PCNB has been found to initiate carcinogenic activity in initiation-promotion studies (Searle, 1966), but not when given as a single subcutaneous injection to B6C3F1 or B6AKF1 mice (BRL, 1968b). In vitro, PCNB was not mutagenic in reverse mutation assays using S. typhimurium (Waters et al., 1982; Haworth et al., 1983) or E. coli (Shirasu et al., 1976; Waters et al., 1982; Moriya et al., 1983) with or without metabolic activation. It did cause reverse mutations in an E. coli WP-2 repair-deficient strain (Clarke, 1971). In an assay for forward mutations in E. coli (Mohn, 1971), the rec assay in Bacillus subtilis (Shirasu et al., 1976) and mitotic recombination in Saccharomyces cerevisiae (Waters et al., 1982), PCNB was not genotoxic. The compound also had no effects in sex-linked recessive lethal assays in Drosophila melanogaster (Waters et al., 1982; Vogel and

Chandler, 1974), a dominant lethal assay in mice (Jorgenson et al., 1976), unscheduled DNA synthesis in human fibroblasts (Waters et al., 1982) or in host-mediated assays in S. typhimurium and Serratia marcescens (Buselmaier et al., 1972).

In a 3-generation study in CD rats, there were no effects on reproductive indices or terata in any generation that were due to the continual administration of up to 500 ppm PCNB in the diet (Borzelleca et al., 1971). No microscopic anomalies were found in the tissues of treated third-generation weanlings. Oral treatment of rats with 200 mg/kg PCNB on gestation days 6-15 led to losses in maternal body weight gain and decreased numbers of liveborn fetuses/litter (Khera and Villeneuve, 1975). There were no dose-related effects on the appearance of terata or postnatal fetal development at $\leq$125 mg/kg (Jordan et al., 1975). In C57B16 (but not AKR) mice, oral administration of 215 or 464 mg/kg PCNB (contaminated with 11% HCB) on days 6-15 of gestation increased the incidences of renal agenesis and, to a lesser extent, microphthalmia, in fetuses (BRL, 1968b). The effects on kidney development were also observed when administration was on gestation days 6-10 but not on days 10-15.

Courtney et al. (1976) conducted a series of studies that were designed to pinpoint the causative factors of the reported renal teratogenicity. Administration of 500 mg/kg PCNB contaminated with 11% HCB to C57B16 mice on gestation days 7-11 increased the incidence of renal and eye malformations and cleft palates in exposed fetuses. There were no teratogenic effects in pups of CD rats dosed with 500 mg/kg contaminated PCNB on a longer treatment schedule. In CD-1 mice dosed daily throughout organogenesis with either 500 mg/kg contaminated PCNB or 100 mg/kg HCB, there were statistically significant increases in the incidences of cleft palate and renal malformations,

respectively. Dosing with 500 mg/kg pure PCNB increased the incidence of clubfoot and fetal mortality. This regimen did not, however, provoke renal agenesis in the offspring and only slightly increased the incidence of cleft palate. The authors concluded that HCB was the etiological agent in renal and ophthalmological terata.

Two-year administration of 0, 5, 30, 180 or 1080 ppm 97.8% PCNB to three male and three female beagles/group, in the diet, had no consistent effect on the body weights, clinical signs, urinalysis results or blood biochemistries (Borzelleca et al., 1971). At the high concentration, the investigators found slightly elevated relative hepatic weights in the high-dose dogs and cholestatic hepatosis and secondary bile nephrosis at 108 and 1080 ppm.

In a long-term study, Swiss mice were exposed to 0, 100, 400 or 1200 ppm PCNB (Van der Heijden and Til, 1974). After 80 weeks, males showed a treatment-related increase in hyperplastic hepatic nodules that was slightly elevated at 100 ppm but markedly increased at 400 and 1200 ppm; this effect was accompanied by hepatomegaly at 400 and 1200 ppm. The appearance of hepatic nodules was not observed in females, although high-dose females were shown to have increased kidney weights. In another study, Wistar rats were given dietary PCNB at 0, 100, 400 or 1200 ppm for 104 weeks (Anonymous, n.d.). At 1200 ppm, relative kidney and liver weights were higher than control values. In both sexes, indications of hepatotoxicity, including enlarged centrilobular hepatocytes, fatty centrilobular metamorphosis and single cell necrosis were slightly elevated at the 100 ppm level and markedly elevated at the higher concentrations. Female albino rats given technical grade PCNB as a 20% powder for 2 years, up to dietary levels of 23,500 ppm, had sporadic inhibition in growth rates (Finnegan et al., 1958).

There were no treatment-related effects upon mortality rates, hematologies or incidences of tissue damage. These investigators also found hepatocellular enlargement and pale-staining cytoplasm in groups of three dogs exposed for 1 year to 25-1000 ppm PCNB in the diet. Two strains of mice given 1298 ppm "contaminated" PCNB in the diet for 77 weeks had increased incidences of pneumonia and lymphoid infiltration, relative to controls (BRL, 1968a). In a more comprehensive bioassay, the NCI (1978) found that TWA concentrations of 10,064-14,635 ppm dietary PCNB led to a "hunched" appearance and abdominal urine stains in male and female rats. At these dietary levels and at lower levels (5417-7875 ppm), there were inhibitions in weight gain relative to controls. The hunched appearance also occurred in treated mice, and female mice treated with up to 9000 ppm (TWA = 8187 ppm) PCNB showed an inhibition of weight gain. No treatment-related effects or nonneoplastic tissue pathology were noted.

In a repeat study using B6C3F1 mice, there was a dose-related decreased body weight gain, increased mortality and increased incidence of ovarian abscesses in females given 2500 and 5000 ppm in the diet for 103 weeks (NTP, 1986). The increased mortality was attributed to a combination of the toxicity of PCNB and the ovarian infections. It was suggested that the PCNB treatment predisposed the female mice to infection.

Fytzias-Danielidou (1975) administered a 75% pure suspension of PCNB to Wistar rats by gavage, 6 days/week for up to 32 weeks. Serial sacrifices were made every 8 weeks. Exposure to 400 mg/kg, and to a lesser extent 200 mg/kg led to increases in mortality, transient inhibitions in weight gain, polyuria, hepatocellular necrosis, hepatomegaly and degeneration of the renal tubular epithelium. There were no changes in hematological parameters.

A number of animal studies involving subchronic oral exposure to PCNB have been conducted. Koegel et al. (1979b) found no effects of 2 ppm dietary PCNB, given over 70-80 days, on four rhesus monkeys, relative to historic controls. In a 13-week oral dosing experiment, Finnegan et al. (1958) noted mortality and morbidity in rats given a dietary concentration of 5000 ppm PCNB (as a 20% dust), and inhibition in growth rate in males given 2500 ppm. At lower concentrations, there were increases in relative liver and kidney weights. The NCI (1978) observed body weight gain depressions and mortality in rats exposed to $\geq$21,500 ppm PCNB and in mice exposed to 4640 (males) or 10,000 (females) ppm for a 6-week period. NTP (1986) found significantly increased relative liver weights in male mice treated at $\geq$2500 ppm and females at $\geq$5000 ppm PCNB in the diet for 13 weeks. Mice of both sexes were emaciated at $\geq$20,000 ppm. The 40,000 ppm level was fatal to female mice and resulted in lymphoid depletion. Finally, Kawano et al. (1979) noted evidence of increased hepatic microsomal activity in rats given 2000 ppm PCNB, of an unknown purity, for 3 weeks.

Acute oral LD_{50} values for PCNB in rodents range from 1.65 to >12 g/kg. The technical grade material has been found to be more hazardous than the wettable powder (Finnegan et al., 1958; Borzelleca et al., 1971; Renner, 1980; Cholakis et al., 1981). Schumann and Borzelleca (1978) reported methemoglobinemia in rats after oral administration of PCNB, an effect not observed by Koegel et al. (1979b) in rhesus monkeys. Dose and species differences were the two most likely reasons for these discrepancies.

Although the evidence indicates that relatively pure PCNB (<1% HCB) did not induce hepatoma in B6C3F1 mice (NCI, 1978; NTP, 1986), albino Swiss female mice fed relatively pure PCNB with HCB contamination of 2.7%, developed statistically significant increased incidences of subcutaneous

fibromas and fibrosarcomas. From these data a q_1^* for humans of 3.93×10^{-2} $(mg/kg/day)^{-1}$ was derived. Another bioassay with 11% HCB contamination shows a hepatoma response in male B6AKF1 and female B6C3F1 mice. These data were used to derive a human q_1^* of 0.26 $(mg/kg/day)^{-1}$. Since the potency of the tested PCNB compounds seems related at least in part to the level of HCB contaminant, any statements about risk levels derived from the available data must be referenced to the purity of PCNB. Arbitrarily, the bioassay with the highest level of PCNB contamination will be used to derive drinking water levels and RQ "F" factors. The concentrations in water associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 1.33×10^{-3}, 1.33×10^{-4} and 1.33×10^{-5} mg/ℓ. An "F" factor of 1.4 $(mg/kg/day)^{-1}$ was also derived. The limited evidence in animals and lack of evidence in humans indicate that PCNB is an EPA Group C chemical. Therefore, PCNB has a LOW hazard ranking under CERCLA. At 180 ppm (2.7 mg/kg/day), dogs had cholestatic hepatosis and bile nephrosis. An RQ of 1000 was also derived based on the liver pathology in dogs at 180 ppm in the study by Borzelleca et al. (1971).

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LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
BUN	Blood urea nitrogen
bw	Body weight
CAS	Chemical Abstract Service
CS	Composite score
DNA	Deoxyribonucleic acid
GC	Gas chromatograph
GPT	Glutamic pyruvic transaminase
K_{oc}	Soil sorption coefficient standardized with respect to organic carbon
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients (and all other subscripted dose levels)
LD_{50}	Dose lethal to 50% of recipients
LDH	Lactic dehydrogenase
MED	Minimum effective dose
MTD	Maximum tolerated dose
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
RBC	Red blood cell
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value

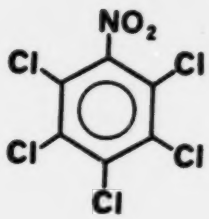
LIST OF ABBREVIATIONS (cont.)

SAP	Serum alkaline phosphatase
SCE	Sister-chromatid exchange
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TLC	Thin-layer chromatography
TWA	Time-weighted average
UV	Ultraviolet
WBC	White blood cell
w/w	Weight per weight

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

The chemical commonly called pentachloronitrobenzene is also known as quintozene and PCNB. Some of the trade names for this chemical are Terrachlor●, Brassical●, Folosan● and Tritison● (Worthing, 1983). The structure, empirical formula, molecular weight and CAS Registry number for PCNB are as follows:



Empirical formula: $C_6Cl_5NO_2$

Molecular weight: 295.34

The CAS Registry number for this compound is 82-68-8.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Pure PCNB forms colorless crystals (Worthing, 1983), but commercial PCNB may have a light-yellow to cream color, with a musty odor (Hartley and Kidd, 1983; Hawley, 1981). It is almost insoluble in water but is soluble in aromatic solvents, ketones, chlorinated hydrocarbons and carbon disulfide (Hartley and Kidd, 1983). Some of the physical properties of PCNB are listed below:

Melting point:	146°C	Worthing, 1983
Boiling point:	328°C (with slight decomposition)	Hartley and Kidd, 1983
Density at 25°C:	1.718	Worthing, 1983

Vapor pressure		
at 25°C:	1.33×10^{-4} mm Hg	Wang and Broadbent, 1972
	1.23×10^{-4} mm Hg	Hartley and Kidd, 1983
at 20°C:	0.53×10^{-4} mm Hg	Hartley and Kidd, 1983
Water solubility		
at 20°C:	0.44 mg/ℓ	Hartley and Kidd, 1983; Korte et al., 1978
Log K_{ow}:	4.64 (recommended value)	Hansch and Leo, 1985
Henry's Law Constant at 20°C:	4.7×10^{-5} atm·m³ mol^{-1} (estimated from vapor pressure and water solubility data)	
Conversion factor at 20°C:	1 ppm = 12.3 mg/m³	

PCNB is reasonably stable when heated (Hartley and Kidd, 1983) because it undergoes slight decomposition at 328°C. The compound is also stable in mildly acidic media. In strong alkaline solutions, PCNB undergoes hydrolysis with the formation of nitrite and pentachlorophenol (Hartley and Kidd, 1983). The nitro group of PCNB is susceptible to reduction with strong reducing agents (Hartley and Kidd, 1983).

1.3. PRODUCTION DATA

Both SRI (1984) and Hartley and Kidd (1983) reported that Olin Corp., Leland, MS, was the only manufacturer of PCNB in the United States at the end of 1983, but production volume data for this compound during that or previous years are not available. Manufacturing information from recent sources (USITC, 1985; SRI, 1985) does not list PCNB, suggesting that this chemical is no longer commercially produced in the United States. PCNB is produced by reacting pentachlorobenzene with fuming nitric acid (Hawley, 1981). Technical grade PCNB is $\leq$98.5% pure (Worthing, 1983), typically containing 97.8% PCNB, 1.8% HCB, 0.4% tetrachloronitrobenzene and <0.1% pentachlorobenzene (U.S. EPA, 1985a).

1.4. USE DATA

The primary usage of PCNB is as a soil fungicide for a wide variety of crops and in seed treatment (Worthing, 1983; Hartley and Kidd, 1983). This compound has not been used for nonagriculture purposes in the United States in recent years (U.S. EPA, 1985a).

1.5. SUMMARY

Pure PCNB is a colorless crystalline solid (Worthing, 1983). The commercial product may have a light-yellow to cream color with a musty odor (Hartley and Kidd, 1983). It is practically insoluble in water but is soluble in a number of organic solvents. The compound is reasonably stable but may undergo hydrolysis in a strong alkaline medium (Hartley and Kidd, 1983). In 1983, Olin Corp., Leland, MS, was the only manufacturer of PCNB in the United States (SRI, 1984; Hartley and Kidd, 1983). No data for U.S. production volume for this chemical are available, but recent production source data (USITC., 1985; SRI, 1985) suggest that this chemical is no longer commercially produced in the United States. The primary usage of PCNB is as a soil fungicide for a wide variety of crops and in seed treatment (Worthing, 1983; Hartley and Kidd, 1983).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

Sufficient information to evaluate the fate and transport of PCNB in water is not available in the literature. A few investigators studied the photolysis of PCNB in solution and in coated form (Kotzias et al., 1979; Crosby and Hamadmad, 1971; Korte and Klein, 1982). When 50 ppb of PCNB coated on dry silica gel was irradiated for 17 hours with light of wavelengths >290 nm from a photoreactor, 43% of the compound photomineralized to CO_2 (Kotzias et al., 1979; Korte and Klein, 1982). The degradation of the chemical was much slower in sunlight compared with its photomineralization with lights from the photoreactor. For example, while 55.8% mineralization occurred for benzene in 6 days with light from the photoreactor, only 9.7% mineralized in 30 days with sunlight irradiation (Korte and Klein, 1982). The UV (wavelength = 253.7 nm) irradiation of PCNB in hexane or methanol solutions resulted in photodegradation of the compound with the formation of reductive dechlorination products. Exposure of the compound to sunlight for 7 days did not produce significant decomposition products (Crosby and Hamadmad, 1971) and, therefore, it was concluded that photolysis of PCNB in natural sunlight may not be important.

Several microorganisms, including filamentous fungi (Aspergillus niger, Penicillium frequentons) and actinomycetes (Nocardia sp., Streptomyces aureofaciens) were shown to degrade PCNB (Chacko et al., 1966; Kobayaski and Rittmann, 1982). The degradation of PCNB with mixed cultures of microorganisms has also been studied. When PCNB was incubated with municipal activated sludge at 25°C, 7% of the compound mineralized in 5 days (Korte and Klein, 1982; Freitag et al., 1979). Studies pertaining to the biodegradation of PCNB in natural water could not be located in the available

literature as cited in the Appendix. Degradation studies with natural soil (Wang and Broadbent, 1972), however, indicate that microbial degradation of PCNB is not an important process in soils and may not be an important process in natural water either.

Schauerte et al. (1982) estimated the half-life in the water phase to be 118 days when 50 µg/ℓ PCNB was added to small experimental ponds. The rapid decrease of PCNB in the water was attributed to volatilization from water and by sorption to seston and biota, followed by sedimentation as detritus. The residue level in the sediment showed an initial increase to 3 mg/kg following application and then decreased to 0.1 mg/kg within 1 year. This decrease in concentration in the sediment was attributed to evaporation into the atmosphere, dissolution in water, biodegradation and slight leaching into soil. The study of Schauerte et al. (1982), however, provided neither direct evidence of volatilization of PCNB from water nor data to estimate the relative distribution of added PCNB in different phases in water. The extent of volatilization of PCNB from water can be qualitatively predicted from its estimated Henry's Law Constant of 4.7×10^{-5} atmos·m^3 mol^{-1} and the volatility characteristics associated with that range (Lyman et al., 1982). Based on this method, significant volatilization of PCNB from water would be expected. Similarly, from the measured distribution coefficient of 4500 for PCNB between sludge and the water phase (Freitag et al., 1979), it is likely that sorption onto sediment will be a major removal mechanism for PCNB from water.

The bioaccumulation of PCNB in aquatic organisms has been reported by Korte et al. (1978), giving a BCF range of 950-1130 for PCNB in the golden orfe, Leucisens indus melanotus. A BCF ranging from 260-590 was reported for PCNB in rainbow trout, Salmo gairdneri, by Oliver and Niimi (1985). It appears that PCNB will moderately bioaccumulate in aquatic organisms.

2.2. AIR

Pertinent data regarding the fate and transport of PCNB in air could not be located in the available literature as cited in the Appendix. Considering the chemical stability of this compound, atmospheric abiotic reactions are not likely to significantly remove PCNB from the atmosphere. The rate constant value for OH radical reaction with PCNB, a likely significant atmospheric reaction, is not available in the literature. The rate constant for nitrobenzene reaction with OH radicals is given as 0.21×10^{-12} cm^3 $molecule^{-1}$ sec^{-1} (Alkinson, 1985). Substitution of five chlorines into the benzene ring of nitrobenzene will decrease the reaction rate with OH radicals compared with the rate with nitrobenzene. If the value of 0.21×10^{-12} cm^3 $molecule^{-1}$ sec^{-1} is taken as the upper limit of reaction rate constant for OH radical reaction with PCNB and the concentration of OH radical in the atmosphere is assumed to be 10^6 molecules cm^{-3}, the lowest possible atmospheric half-life for PCNB that is due to reaction with OH radical will be 40 days. Therefore, this reaction may not be significant in the loss of PCNB in the atmosphere. Photolysis studies (see Section 2.1.) imply that photolysis may not be significant in the atmosphere. No experimental or estimated data regarding the removal of PCNB from the atmosphere through physical processes were available in the literature. Because of the low water solubility, removal of PCNB through wet precipitation may also not be significant. Precipitation of particulate PCNB from the atmosphere will depend on its particle size and density. In the absence of such data, it may be qualitatively predicted that some precipitation of particulate PCNB from the atmosphere will occur.

2.3. SOIL

Since PCNB is used as a soil fungicide, its fate and transport in soil is more thoroughly studied than in any other media. Since PCNB is stable at acidic and weakly basic pH (Hartley and Kidd, 1983), hydrolysis of this compound is not likely to occur in soil. Photolysis of PCNB in soil is likely to be insignificant since the compound coated on silica gel and in hexane/methanol solution failed to produce significant photodegradation in natural sunlight (Korte and Klein, 1982; Crosby and Hamadmad, 1971). When a stream of water-saturated air was passed through fine sandy soil containing PCNB (25°C), 80% of the applied PCNB was lost from the soil in 10 months (Casley, 1968). Removal in the air stream accounted for 62% of PCNB removal. The remaining 18% could be attributed to microbial and chemical degradation. Data from sterilized soil studies, however, indicate that most of the 18% loss could be attributed to biodegradation. Therefore, chemical/photochemical reaction have very little effect on the degradation of PCNB from soil.

Several pure cultures of microorganisms including actinomycetes (Nocardia, Streptomyces) and fungi, Aspergillus, can degrade PCNB under aerobic conditions (Chacko et al., 1966; Bourquin, 1973). The biodegradation of PCNB under anaerobic conditions was much faster than under aerobic conditions (Ko and Farley, 1969). For example, 99% of applied PCNB was lost from submerged soil in 3 weeks of incubation, compared with an 18% loss from moist soil. It was also shown that 56% of PCNB loss from submerged soil was due to biodegradation, while the residual 44% loss was due to nonbiological processes including volatilization. In anaerobic soils, PCNB is microbially converted to pentachloroaniline, whereas in aerobic soils it is converted

primarily to pentachlorothioanisole and to smaller amounts of pentachloroaniline and pentachlorobenzene (Dejonckheere et al., 1975b; Murthy and Kaufman, 1978; Alexander, 1981; Buser and Bosshardt, 1975; Devos et al., 1974).

Caseley (1968) observed loss of PCNB up to 62% by volatilization from a 50% (w/w) moist soil in 10 months. Losses by volatilization increased as soil moisture content increased; this was due presumably to its displacement from adsorption sites by water. A loss of ~30% PCNB through volatilization was observed from a soil of 20% (w/w) moisture content. Similar losses from volatilization were also noted by Murthy and Kaufman (1978), and Wang and Broadbent (1972) concluded from their studies that volatilization is a major route of PCNB loss from soil.

Concerning the fate of PCNB in terrestrial laboratory ecosystems, Cole and Metcalf (1977) reported the detection of 34% of the applied dose in air, 2% in animals (earthworms, slugs, pillbugs, caterpillars and voles) and 64% in combined soil and plant (corn) at the end of 20 days. Gile and Gillet (1979), on the other hand, reported the following distribution of applied dose in different parts of the ecosystem after 45 days: 24% in air, 0.06% in animals, 16% in plants, 59% in soil, 0.3% in groundwater and 0.7% in miscellaneous phases. Both these studies show the persistence of PCNB in soils.

The persistence of PCNB in a variety of field and laboratory soils was studied by Beck and Hansen (1974), who reported the average half-life to be 434 days. Wang and Broadbent (1972) estimated the following half-lives for PCNB in three different soils: 4.7 months for fine sandy loam (0.34% organic carbon), 7.6 months for clay (1.83% organic carbon) and 9.7 months for peaty muck (22.43% organic carbon).

Cairns et al. (1983) and Dejonckheere et al. (1975b) studied the metabolism of PCNB in plants and reported that PCNB is primarily metabolized to pentachloroaniline and to pentachlorothioanisole as a minor product. Evidence of very restricted (~3% on the average) translocation of PCNB and its metabolites from the roots to the foliar tissue was reported by Cairns et al. (1983).

The mobility of PCNB in silty clay loam soil determined by soil TLC was studied by Helling et al. (1974). PCNB was reported to be immobile, with an R_f value of 0. From the terrestrial laboratory ecosystem study with artificial soil consisting of 10% (wt.) Jiffy Mix Plus, 45% sea sand and 45% sillite clay, Gile and Gillett (1979) reported some vertical distribution of PCNB. Forty-five days following the application of 0.64 kg/ha PCNB, no residues of the parent compound, metabolite or bound residues were detected beyond 15 cm of the soil layer. The probability of PCNB leaching through soils can also be predicted from its water solubility and K_{oc} data. From the equation, log K_{oc} = 0.55 log S + 3.64 (S = solubility in mg/ℓ) (Lyman et al., 1982), the K_{oc} value is estimated as 6855. Combining this K_{oc} value with the water solubility of 0.44 mg/ℓ, it is predicted that PCNB will be moderately mobile in soil.

2.4. SUMMARY

The fate of PCNB in water has not been comprehensively studied. Only qualitative data regarding fate and transport in water are available. The half-life of PCNB in the water phase was estimated to be 1.8 days. The two processes reported to be most responsible for the rapid decrease in PCNB concentration in water were volatilization and sorption to seston and biota, followed by sedimentation as detritus (Schauerte et al., 1982). Neither biodegradation nor photolysis appears to be a significant process for the loss of PCNB from water (Crosby and Hamadmad, 1971; Schauerte et al., 1982).

The BCFs for PCNB in the golden orfe, Leucisens idus melanotus, and in rainbow trout, Salmo gairdneri, were reported to be 950-1130 and 260-590, respectively (Korte et al., 1978; Oliver and Niimi, 1985). It therefore appears that PCNB will moderately bioaccumulate in aquatic organisms.

Pertinent data regarding the fate and transport of PCNB in air could not be located in the available literature as cited in the Appendix. Based on its physical properties and its behavior in other media, it would appear that PCNB will persist in the atmosphere because no known chemical/photochemical processes significantly degrade this chemical. Precipitation of particulate PCNB, especially of larger particle size and higher particle density, may remove some PCNB from the atmosphere.

PCNB is persistent in soils. The two processes that are important in the loss of PCNB from soils are volatilization and biodegradation; biodegradation is more rapid in soils under anaerobic conditions than under aerobic conditions (Ko and Farley, 1969; Casley, 1968; Gile and Gillett, 1979; Cole and Metcalf, 1977). The average half-life of PCNB in a variety of field soils was reported to be 434 days (Beck and Hansen, 1974). Another study reported the half-lives for PCNB in sandy loam, clay and peaty muck soils as 4.7, 7.6 and 9.7 months, respectively (Wang and Broadbent, 1972). PCNB is expected to be moderately mobile in soils (Gile and Gillett, 1979). Evidence of very restricted (about 3% on the average) translocation of PCNB and its metabolites from the roots to foliar tissue in plants was reported by Cairns et al. (1983).

3. EXPOSURE

3.1. WATER

Pertinent data regarding the levels of PCNB in ambient or drinking water could not be located in the available literature as cited in the Appendix. The U.S. EPA STORET database contains 120 entries on the analysis of PCNB in water samples, which include both ambient and aqueous effluent samples. The maximum and minimum PCNB concentrations in these samples were 1.0 and 0.01 ppb, respectively, with a mean value of 0.4 ppb.

3.2. AIR

Although the atmosphere surrounding an application site is likely to be contaminated with PCNB and PCNB may also originate in the atmosphere from the volatilization of applied materials, no data on levels in ambient air are available in the literature. The atmospheric level of PCNB under one occupational condition was reported by Yeboah and Kilgore (1984), who measured 1.56 $\mu g/m^3$ of PCNB inside the ambient air of a commercial pesticide building.

3.3. FOOD

Since PCNB is applied as a fungicide on several agricultural crops, its level in food items has been more thoroughly studied than its level in any other media. Dried sugar beet samples grown in Belgium where PCNB was used as a fungicide were found to contain levels of <1 mg/kg (Valange and Henriet, 1974); PCNB was also detected in samples of lettuce grown in Belgium (Valange, 1974). Treatment of red cabbage with the recommended dosage of PCNB resulted in a residue level of 0.1-0.8 mg/kg on the inner leaves before and 0.2-0.3 mg/kg after washing; the corresponding values for treated white cabbage were 0.6-1.0 and 0.3-0.8 mg/kg, respectively (Feuersenger, 1961). Wheat fields in southern Finland treated with 4-6 kg

air/ha of PCNB left a residue of 0.14 mg/kg of PCNB in the wheat (Rautapaa et al., 1977). The level of PCNB in food samples in the United States was determined by the FDA by analyzing total diet samples for a number of pesticides and trace metals. These samples (for adults) were divided into 12 classes of food categories: dairy products; meat, fish and poultry; grain and cereal products; potatoes; leafy vegetables; legume vegetables; root vegetables; garden fruits; fruits; oil, fats, and shortening; sugar and adjuncts; and beverages including drinking water. Of samples from different regions in the United States from 1964-1965, only "fruits" contained PCNB, at a level of 0.003 mg/kg (Duggan et al., 1966). In every survey year from 1971-1978, PCNB was detected in the class of oils, fats and shortening, with levels ranging from trace to 0.006 mg/kg (Johnson et al., 1981a, 1984a; Johnson and Manske, 1976, 1977; Manske and Johnson, 1975, 1977; Podrebarac, 1984a). PCNB was also detected in sugar and adjuncts composites at trace levels in 1977-1978, in fruits composite at an average trace level in 1974-1975 and in potatoes composite at an average trace level in 1973-1974 (Manske and Johnson, 1977; Johnson and Manske, 1977; Podrebarac, 1984a). If it is assumed that the maximum level of PCNB in oils, fats and shortening is 0.006 mg/kg (Johnson et al., 1984a), the maximum daily intake of PCNB will be 0.4 µg. The FDA also sampled infant and toddler diets in the United States from 1975-1976, 1976-1977 and 1977-1978 (Johnson et al., 1981b, 1984b; Podrebarac, 1984b). PCNB was detected only in oils and fats composites at levels between 0 and 0.015 mg/kg. The daily intake of PCNB from Canadian total diet samples was estimated to range between 0 and 0.4 µg/person/day; this fungicide was detected only in leafy vegetables (McLeod et al., 1980).

3.4. SUMMARY

Pertinent data regarding the levels of PCNB in ambient and drinking water could not be located in the available literature as cited in the Appendix. U.S. EPA's STORET database contains 120 entries on the analysis of PCNB in ambient water and aqueous effluent samples. Maximum and minimum PCNB concentrations in these samples were 1.0 and 0.01 µg/ℓ, respectively, with a mean value of 0.4 µg/ℓ.

Pertinent data regarding the ambient atmospheric level of PCNB could not be located in the available literature as cited in the Appendix. The concentration of PCNB in the ambient air of a commercial pest control building was reportedly 1.56 µg/m³ (Yeboah and Kilgore, 1984).

In the foods consumed by U.S. adults, infants and toddlers, PCNB residues have been detected mainly in the oils, fats and shortening group of foods (Johnson et al., 1981a,b, 1984a, Johnson and Maske, 1976; Maske and Johnson, 1975; Podrebarac, 1984a,b). Occasionally, PCNB has been detected in sugar and adjuncts, fruits and potatoes (Manske and Johnson, 1977; Johnson and Manske, 1977; Podrebarac, 1984a). On the basis of the assumption of a maximum PCNB content of 0.006 mg/kg (Johnson et al., 1984a) in oil, fats and shortening composite and a daily adult consumption of 0.070 kg/day of this category of food (Johnson et al., 1984a), the maximum daily adult intake of PCNB in the United States has been estimated to be 0.4 µg. The Canadian daily PCNB intake has been reported to vary between 0 and 0.4 µg (McLeod et al., 1980).

4. PHARMACOKINETICS

4.1. ABSORPTION

PCNB was administered as an aqueous solution to rabbits at oral doses of 1, 2 or 3 g and urine and feces were collected for 72 hours (Betts et al., 1955). Mean percentages of the amounts of PCNB recovered in the feces ranged from 46-62%, leading the authors to conclude that PCNB was largely unabsorbed. Betts et al. (1955), however, did not measure biliary excretion. Korte et al. (1978) administered three daily oral doses of ^{14}C-PCNB by gavage to male rats. The dose of PCNB administered was not clear; however, it appeared that the gavage dose was equivalent to a dietary concentration of 1 ppm. Radioactivity in the urine and feces was measured daily, and the rats were killed on day 8. Urinary excretion of radioactivity accounted for 8.7% of the total 8-day dose and fecal excretion for 89.9%. For the 5-day elimination phase, the corresponding values were 3.7 and 32.3%.

Koegel et al. (1979a,b) gave single oral doses of ^{14}C-PCNB to male and female rhesus monkeys for studies of distribution, uptake and excretion. One monkey received 0.5 mg/kg PCNB dissolved in ethanol, eight received a dose of 2.0 mg/kg PCNB dissolved in aqueous methyl cellulose, and one received 91 mg/kg dissolved in sesame seed oil. Samples of blood, urine and feces were collected for 5-23 days. After the single oral dose of 0.5 mg/kg, a peak blood level of radioactivity was reached in 1.5 hours. This peak was followed by a second maximum after 5-7 hours, which was due to a build-up of metabolites in the blood. Thus absorption was rapid. After a single oral dose of 2.2 mg/kg, ~22-28% of the dose of radioactivity was excreted in the urine and ~10-20% in the feces collected over 24 hours. Since the concentration of radioactivity in the bile at 24 hours was 276 ppm (compared with 2.3 and 1.6 ppm in the liver and kidney, respectively), much

of the fecal excretion was due to biliary excretion. At 48 hours after dosing, the concentration in bile (14 ppm) remained high relative to that of the liver (0.5 ppm) and kidney (0.4), indicating that biliary excretion continued to be a major excretory process. Total excretion of radioactivity at 14 days was 36-39% in urine and 45-46% in feces. Only 7.4% of the 2 mg/kg dose was excreted as unchanged PCNB in feces indicating that absorption was extensive. After a dose of 91 mg/kg, a total of only 4.3% of the dose was excreted unchanged in the feces, indicating equal or greater absorption after the higher dose.

4.2. DISTRIBUTION

Koegel et al. (1979a,b) studied the tissue distribution of orally administered ^{14}C-PCNB in two rhesus monkeys given a single oral dose of 2 mg/kg and in two additional monkeys given the equivalent of a dietary level of 2 ppm in sucrose pellets for 70 days. In the single dose study, one male monkey was sacrificed 24 hours after dosing and the second 48 hours after dosing. At 24 hours, measureable but low levels (0.1-0.7 ppm) of radioactivity were found in the adrenal cortex, thymus, bone marrow, heart, muscle, lymph nodes, fat, brain and blood (plasma and erythrocytes). The level in the kidney was 1.6 ppm and in the liver 2.3 ppm. At 48 hours, levels were 0.5 and 0.4% in the liver and kidney, respectively, and nondetectable or $\leq$0.4 ppm in all other tissues. A similar distribution, but low levels of radioactivity was found in monkeys sacrificed 1 day after the 70-day dosing schedule. Ten days after the last dose, surviving monkeys had excreted ~95% of the total dose.

In the previously described study by Korte et al. (1978) (see Section 4.1.), 5 days after the last dose, the rats were sacrificed and radioactivity was measured in liver, lung and subcutaneous fat. No detectable levels

(detection limit = 0.01%) of radioactivity were found in any of these tissues. In rats given lifetime exposure to various dietary concentrations of PCNB, and in dogs exposed for 2 years, there was no detectable parent compound in any tissue at necropsy, although low levels of the metabolites, pentachloroaniline and pentachlorothioanisole (also called methylpentachlorophenyl sulfide) were found in muscle, liver, kidney and fat of rats and kidney, brain, liver and fat of dogs (Borzelleca et al., 1971).

Courtney et al. (1976) administered PCNB orally to gravid C57Bl/6 mice on days 7-10 or 7-11 of gestation. All adult mice and fetuses (combined with amniotic fluid and placenta) were examined for PCNB, pentachloroaniline and methyl pentachlorothioanisole content 24 hours after the last dose. Trace levels of the parent compound and its metabolites were found in the fetuses. In single-dosed males and nongravid females, tissue concentrations of PCNB in the testes, uterus and ovaries were highest at 2 hours after dosing and had declined by 48 hours.

Villeneuve and Khera (1975) found no accumulated PCNB in maternal or fetal tissues from Wistar rat dams given PCNB (95% pure) orally from days 6-15 of gestation and sacrificed on day 22. All tissues examined revealed accumulation of the contaminated HCB. These results were supported by a study in which mass chromatographic analysis of the placentas of Japanese women showed that the concentrations of HCB correlated with those in the milk and umbilical cord blood (Ando et al., 1985). Ando et al. (1985) stated that one of the main sources of HCB exposure in Japan is derived from PCNB and other commercial pesticides.

4.3. METABOLISM

PCNB metabolism involves a large number of reactions that lead to a wide variety of intermediates and end products (Renner, 1981; Renner and Nguyen,

1984). The appearance of these metabolites is both species and dose-dependent. Betts et al. (1955) orally dosed rabbits with 1, 2 or 3 g PCNB and ascertained the presence of mercapturic acid and pentachloroaniline in the urine collected for 72 hours. At 3 g, 4% of the administered dose was mercapturic acid and 14% was pentachloroaniline. Subsequent precipitation and extraction of the crude mercapturic acid fraction gave N-acetyl-S-pentachlorophenyl-L-cystine.

Results of a GC analysis of tissues from dogs that received 2-year dietary levels of PCNB at 5, 30, 180 or 1080 ppm PCNB were reported by both Kuchar et al. (1969) and Borzelleca et al. (1971). The technical grade sample, also known as Terraclor®, contained 1.8% HCB as an impurity. Analysis of muscle, kidney, fat and liver tissue revealed the presence of pentachlorobenzene, pentachloroaniline and pentachlorothioanisole. Large amounts of apparently unmetabolized HCB were found in all tissues, but especially in fat. Male rats given 5, 50 or 500 ppm PCNB for 7 months, then sacrificed, also had the same metabolites and impurity in the fat tissue. These metabolites and HCB were present in the skeletal muscle, liver and kidney only at 500 ppm. In a separate group of males given a control diet for 2 months after 7 months of the PCNB diet, only HCB was detected. Kuchar et al. (1969) found that pentachloroaniline could further react to form glucuronic and sulfuric acid conjugates.

The major urinary products after oral dosing of rats with 5 mg/kg ^{14}C-PCNB were pentachloroaniline (18% of dose), pentachlorocysteine (48%), pentachlorophenol (4%) and pentachlorothioanisole (0.2%) (O'Grodnick et al., 1981).

Groups of male and female C57Bl/6 mice receiving 4-5 daily oral doses of PCNB were studied by Courtney et al. (1976) for excretion of parent compounds and metabolites. The dose used was 500 mg/kg/day and after each of the first three doses, pentachloroaniline was found to be the major component of urine, although the major component in the feces was the parent compound. After the third dose, the volume of urinary excretion of PCNB and pentachloroaniline was ~4-7 times greater in males than in females. Pentachlorothioanisole was found as a minor metabolite in both the urine and feces.

The urinary and fecal metabolites of PCNB in rhesus monkeys administered a single oral dose of 2 or 91 mg/kg, or given the equivalent of a dietary level of 2 ppm for 70 days were identified by GC-MS by Koegel et al. (1979c). The major metabolites and the percentages found in the urine and feces are presented in Table 4-1. The metabolites following a single oral dose of 2 mg/kg and the 2 ppm diet are remarkably similar, qualitatively and quantitatively. Following the dose of 91 mg/kg, however, relatively more pentachlorophenol and relatively less pentachloroaniline were excreted in the urine. In addition, six more identified and five unidentified metabolites were found following the high dose; however, lower yields of radioactive material following the lower doses may account for the difference. Unchanged PCNB was found only in the feces. The metabolites found in the bile were identified as mercapturic acid conjugates of pentachloroaniline, pentachlorobenzene, pentachlorothioanisole and 2,3,4,5-tetrachloroaniline.

Koegel et al. (1979c) proposed two major metabolic pathways for PCNB in monkeys. In the first, reduction of the nitro group results in pentachloroaniline. In the second, PCNB forms a conjugate with glutathione. The

TABLE 4-1

Percentage of PCNB and Metabolites in Extracts of Urine and Feces of Monkeys*

Metabolite or PCNB	Percent of Urinary Extract	Percent of Fecal Extract
Single oral dose of 2 mg/kg:		
Pentachloroaniline	55.2	66.0
Pentachlorobenzene	11.7	1.0
Pentachlorophenol	12.2	0
Pentachlorothioanisole	9.7	6.2
Pentachlorothiophenol	1.4	0.2
2,3,4,5-Tetrachloroaniline	0.1	0.1
Bis-methylmercaptotetrachlorobenzene	9.7	7.1
Tetrachloroaminothioanisole	0	3.1
Unchanged PCNB	0	16.3
Single oral dose of 91 mg/kg:		
Pentachloroaniline	36.0	66.2
Pentachlorobenzene	11.9	1.1
Pentachlorophenol	17.5	0
Pentachlorothioanisole	10.3	6.2
Pentachlorothiophenol	1.4	0.2
2,3,4,5-Tetrachloroaniline	1.0	0.1
2,3,4,6-Tetrachlorophenol	1.8	0
Tetrachlorothioanisole	3.4	0
Tetrachloroaminothioanisole	1.6	3.1
Tetrachloroaminophenylmethylsulfoxide	0.3	2.0
Tetrachlorophenylmethylsulfoxide	3.7	0
Bis-methylmercapto-aminotrichlorobenzene	0.1	0
Bix-methylmercapto-tetra-chlorobenzene	9.2	7.1
Pentachlorophenylmethylsulfoxide	>0.01	0
Unchanged PCNB	1.0 0	12.9
2 ppm in diet for 70 days:		
Pentachloroaniline	55.4	70.6
Pentachlorobenzene	11.8	0.5
Pentachlorophenol	12.2	0
Pentachlorothioanisole	9.8	6.0
Pentachlorothiopehnol	1.0	0.1
2,3,4,5-Tetrachloroaniline	0.1	0.01
Bis-methylmercapto-tetrachlorobenzene	9.7	9.3
Tetrachloroaminothioanisole	0	0.09
Unchanged PCNB	0	13.4

*Source: Koegel et al., 1979c

conjugate is metabolized further to pentachlorothioanisole, pentachlorothiophenol and PCNB. Conjugation twice with the same molecule of PCNB could give rise to bis-methylmercapto-tetrachlorobenzene and the other tetrachlorometabolites. Conjugation combined with reduction could result in tetrahloroaminothioanisole, tetrachloroaniline, tetrachloroaminophenol or tetrachloroaminothiophenol. Koegel et al. (1979c) proposed that similar pathways operate in rats, and attributed the failure of Borzelleca et al. (1971) to identify more metabolites in rats to differences in procedures rather than to species differences.

4.4. EXCRETION

In the experiments of Koegel et al. (1979a,b), 50.3% of the radioactivity following a single oral dose of 0.5 mg/kg ^{14}C-PCNB to monkeys was excreted in the urine (28.5%) and the feces (21.8%) in 24 hours. At 5 days after dosing, 81.4% of the radioactivity was excreted (39.9% in urine, 41.5% in feces). As discussed in Section 4.1., much of the fecal excretion was due to biliary excretion. Following a dose of 2 mg/kg, total excretion was 81-85% in 14 days. After a single dose of 91 mg/kg, excretion was slower with ~50% excreted in 4 days and 59.5% excreted in 20 days. The plasma elimination curve of radioactivity was log-linear, indicating first-order elimination with a half-life of 6 days. In the 70-day feeding experiment, the excretion curve was parallel to the cumulative dose curve from day 30 on, indicating that steady-state was reached.

O'Grodnick et al. (1981) provided evidence for sex differences in excretion patterns after oral dosing with PCNB. Osborne-Mendel rats were administered 5 mg/kg (10 µCi) ^{14}C-PCNB in cottonseed oil, and the urine and feces were collected and analyzed daily for 6 days. After this period, male rats excreted 10.5% of the radioactivity in the urine and 72% in the feces.

Females excreted 33.4% in the urine and 53.7% in the feces. There were no sex-related differences in tissue retention.

4.5. SUMMARY

Pharmacokinetic and metabolism studies of PCNB indicate that it is rapidly and extensively absorbed from the gastrointestinal tract of rabbits, rats and monkeys (Betts et al., 1955; Korte et al., 1978; Koegel et al., 1979a,b). Following absorption by monkeys and rats, PCNB is rapidly metabolized and the metabolites are widely distributed to tissues but do not accumulate (Koegel et al., 1979a,b; Borzelleca et al., 1971). Courtney et al. (1976) demonstrated that PCNB and its metabolites can cross the placental barrier of mice. Major metabolites in urine and feces of monkeys and rats include pentachloroaniline, pentachlorothioanisole, pentachlorobenzene, pentachlorophenol, pentachlorothiophenol and mercapturic acid conjugates (Borzelleca et al., 1971; Koegel et al., 1979c). Metabolic pathways in monkeys involve reduction of the nitro group to pentachloroaniline and conjugation with glutathione. Conjugation twice with the same molecule of PCNB can lead to bis-methylmercapto-tetrachlorobenzene and other tetrachloro metabolites. Conjugation and nitro reduction combined can lead to tetrachloroamino metabolites. Similar pathways may operate in rats. The metabolites are excreted in the urine and bile. Biliary excretion accounts for the fecal metabolites. Unchanged PCNB was excreted only in the feces of monkeys. The rate of excretion of PCNB and metabolites was dose-dependent: 50% was excreted in 24 hours following a dose of 0.5 mg/kg; 50% was excreted in 4 days following a dose of 91 mg/kg.

5. EFFECTS

5.1. CARCINOGENICITY

An 18-month oral study was conducted in B6C3F1 and B6AKF1 mice of both sexes exposed to PCNB (BRL, 1968a) (Table 5-1). At 1 week of age, 18 mice/sex/strain were started on the test. Until the age of 4 weeks, the mice were gavaged with 464 mg/kg/day PCNB in 0.5% gelatin [contaminated by 11% HCB (Courtney et al., 1976)]. Starting on week 5 and continuing through week 78 of age, the mice received a dietary concentration of 1206 ppm PCNB (BRL, 1968a). The TWA concentration of PCNB administered for 77 weeks was 1298 ppm. Mice were checked daily for morbidity and weighed at weeks 4, 26, 52 and 78. Moribund animals were killed and gross necropsies were performed; mice with grossly observable lesions were then further studied histologically. At the end of 78 weeks, all remaining mice were sacrificed and major organs dissected for histological analysis.

BRL (1968a) included four groups of untreated negative controls of 18 mice/sex/strain and one group of negative controls which received 0.5% gelatin by gavage during weeks 1-4. All negative controls received a standard mouse diet (without PCNB) from weaning until sacrifice.

The investigators found no treatment-related effects on lymphatic leukemia, reticulum cell sarcoma, pulmonary adenoma/carcinoma, hepatic carcinoma with pulmonary metastases, or mammary or skin carcinomas, in either sex or strain. As shown in Table 5-1, there were significant ($p<0.001$) increases in the incidences of hepatomas in female B6C3F1 and male B6AKF1 mice, relative to pooled negative controls. The incidence of these tumors, which BRL (1968a) described as locally invasive with massive involvement of the liver, excluded rare hepatic neoplasms that metastasize

TABLE 5-1

Incidence of Hepatomas in Two Strains of Mice Treated Orally with PCNB (Contaminated with 11% HCB) for 77 Weeks[a,b]

Species/ Strain	Sex	Concentration (ppm)	Tumor Incidence (p value)
B6C3F1	F	0[c]	0/87
	F	1298[d]	4/18 (6×10^{-4})[e]
B6AKF1	M	0[c]	5/90
	M	1298[d]	10/17 (1×10^{-6})[e]

QUALITY OF EVIDENCE

Strengths of Study: Relevant route of exposure for a sufficiently long period of time. Both sexes of mice in two different strains. Several different tissue types studied histologically. Experimentation begun at an early age. Positive controls used.

Weakness of Study: Only one dose level used. Low number of experimental animals. Inadequate statistics. Chemical contamination.

Overall Adequacy: Inadequate.

[a]Source: BRL, 1968a

[b]Treatment schedule: Daily oral administration by stomach tube, 464 mg/kg/day, from weeks 1-4 after birth. Dietary administration from weeks 5-78, 1206 ppm.

[c]Pooled controls, consisting of 79-90 necropsied mice/sex/strain. Pooling was done across four levels of untreated control and one level of controls that received oral gelatin from weeks 1-4. All weaned controls received a standard mouse diet, without PCNB, until sacrifice.

[d]TWA concentration for 77 weeks

[e]By cumulative Fisher exact test, independent analysis at SRC

to the lung. These incidences were similar to those observed after administration of the positive control, ethyl carbamate. Hepatoma incidence did not increase in treated male B6C3F1 or female B6AKF1 mice.

There is, however, sufficient evidence that the contaminant, HCB, is carcinogenic to mice and hamsters (IARC, 1979). Cabral et al. (1978) reported that 10-35% of Swiss mice fed diets containing pure HCB at ≥100 ppm developed liver cell tumors. Dietary administration of pure HCB to hamsters at ≥50 ppm also resulted in dose-related increased incidences of hepatomas (47-87% treated hamsters) and liver hemangioendotheliomas (3-35% treated hamsters) (Cabral et al., 1977).

In a chronic bioassay of the carcinogenic potency of PCNB, NCI (1978) administered dietary PCNB to Osborne-Mendel rats and B6C3F1 mice for 78 weeks. Gas-liquid chromotagraphic analysis indicated a purity of ~97%, with 12 contaminants present. Each sex and species began with 20 control, 50 low-dose and 50 high-dose animals. The low-dose male and female rats were begun at 7500 and 11,000 ppm, respectively. After 13 weeks, because of adverse clinical reactions at the higher doses, these values were lowered to 5000 and 7250 ppm, respectively, for the duration of the study. High-dose male and female rats were initiated at 15,000 and 22,000 ppm, respectively, but were decreased to 10,000 and 14,500 ppm, respectively, at 13 weeks. Because of continued toxicity even at these reduced levels, the NCI (1978) imposed a schedule of a treatment-free week followed by 4 weeks of treatment at 10,000 ppm (males) and 14,500 ppm (females), maintained from weeks 53-78 (males) and 49-78 (females). TWA concentrations for low-dose males and females were 5417 and 7875 ppm, respectively, and for high-dose males and females, 10,064 and 14,635 ppm, respectively. After the 78-week treatment period, all surviving rats were observed for an additional 33-35 weeks.

Controls were fed an untreated standard rat diet for 111-113 weeks before sacrifice.

The low-dose male and female mice were initially administered 1075 and 2320 ppm PCNB and the high-dose males and females with 2150 and 4640 ppm PCNB, respectively (NCI, 1978). Because no toxic effects were found at any concentration, these levels were elevated four times during the 78-week treatment period. TWA doses during treatment were 2606 and 4093 ppm for the low-dose males and females, respectively, and 5213 and 8187 ppm for the high-dose males and females. After the 78-week treatment period, all surviving mice were observed for an additional 14-15 weeks. Controls were fed an untreated mouse diet for 91-92 weeks before sacrifice.

Measurements of body weight, food consumption, clinical effects and tissue masses were made weekly for 10 months and monthly thereafter. Animals were checked daily for mortality, and necropsies performed on all animals that died or were killed when moribund during the study or sacrificed at the end of the study. Microscopic pathological examinations on 30-31 tissue sites were performed.

The NCI (1978) found that a sufficient number of rats survived the 101-week period for analysis of late-developing tumor incidence. Beyond 75 weeks, however, male mice in all groups died at an accelerated rate, so that there were only 25 low-dose, 17 high-dose and 4 controls remaining at terminal sacrifice. A sufficient number of female mice survived for analysis of late-developing tumors.

Fisher exact tests revealed no significant differences in tumor incidences between treated and control animals of either sex or strain. A negative ($p=0.043$) concentration-related trend for mammary fibroadenoma incidence was observed in male rats, by use of the Cochran-Armitage test.

Both low-dose male and high-dose female mice had slightly increased incidences of hepatocellular carcinoma, relative to control values (Table 5-2). Although neither of these incidences was statistically significantly above control rates, a positive Cochran-Armitage trend (p=0.043) was found for the female data. The NCI (1978) necropsied 47 low-dose and 46 high-dose female mice, but performed comprehensive hepatic histopathologies on only 14 and 20, respectively. Apparently, the remaining mice were partially autolyzed. Historical incidence of hepatocellular carcinoma formation in BC63F1 female mice was noted to be 3/380.

Albert (1977) and U.S. EPA (1977) reviewed a preliminary report by Hazleton Laboratories of the NCI (1978) study. In these reviews, the incidence of liver carcinomas in male mice was 2/20 controls, 8/18 low-dose and 4/36 high-dose. The incidence at the low dose was statistically significantly higher than that of the control (p=0.021). The reason for the discrepancy in incidences between the Albert (1977) review and the NCI (1978) final report is not apparent; however, because the NCI (1978) report is the final report, the incidences as reported by NCI (1978) are considered more accurate.

A recently completed repeat study of PCNB in B6C3F1 mice was available as a galley draft (NTP, 1986). In this bioassay, groups of 50 male and 50 female mice were given diets containing PCNB (>99% PCNB; 0.07±0.01% HCB) at 0, 2500 or 5000 ppm for 103 weeks. After 1 week of observation, the mice were killed. Gross and comprehensive histological examination was performed on all mice that died or were killed when moribund during the study or were killed at the end of the study. Based on food consumption data, NTP (1986) calculated that the doses of PCNB were 400 and 1000 mg/kg/day for low- and high-dose males, respectively. There was no effect on the survival of male

TABLE 5-2

Incidence of Hepatocellular Carcinomas in B6C3F1 Mice Treated Orally with 97% PCNB in the Diet for 78 Weeks and Observed an Additional 33-35 Weeks[a,b]

Sex	Concentration (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Tumor Incidence (p value)
M	0	NA	91	2/20 (NS)[d]
	2606[c]	78	92	8/35
	5213[c]	78	92	4/42
F	0	NA	92	0/20 (0.043)[d]
	4093[c]	78	93	0/14
	8187[c]	78	93	3/20

QUALITY OF EVIDENCE

Strengths of Study: Relevant route of exposure for a sufficiently long period of time. Two sexes and strains of rodents used. Comprehensive histopathology. Adequate number of experimental animals. Use of two concentrations.

Weakness of Study: Low control number of animals. Long post-treatment observation period may have obscured tumor incidences. Histology on some tumor sites, including hepatocellular cancer, done on a limited number of samples. Excessive manipulation of dietary concentrations.

Overall Adequacy: Inadequate.

[a]Source: NCI, 1978

[b]Starting number: controls (20), low dose (50) and high dose (50)

[c]TWAs

[d]Significance level for Cochran-Armitage trend test

NA = Not applicable; NS = not significant

mice. In female mice, however, survival of low- and high-dose groups decreased as a combined result of PCNB toxicity and ovarian infections. Final survival in female mice was 30/50 controls, 20/50 low-dose and 15/50 high-dose. No treatment-related neoplastic lesions were observed in any group; however, NTP (1986) stated that the decreased survival of treated females "decreased the sensitivity of the study to determine the presence or absence of a carcinogenic response." Nevertheless, NTP (1986) concluded that under the conditions of the bioassay, there was no evidence of carcinogenicity.

Chronic carcinogenicity bioassays in Swiss mice and Wistar rats have been summarized by Albert (1977). As cited by the U.S. EPA (1977), the mouse study appears in unpublished form as Van der Heijden and Til (1974) and the rat study is an unpublished report by Anonymous (n.d.). The technical grade material used in this study may have been contaminated with as much as 2.7% HCB, although this value is unclear because the sum of all listed ingredients was >100%. The compound was administered in the diet to groups of 100 weanling albino Swiss male and female mice at 0, 100, 400 or 1200 ppm for 80 weeks. Surviving mice were sacrificed after the treatment period, and gross necropsies performed. The liver, lungs and tissues with gross abnormalities (including observable tumors) were further examined microscopically.

Survival time was not greatly affected by PCNB administration. Gross examination revealed an excessive number of skin abscesses and subcutaneous masses in female mice exposed to the high concentration. Histological examination revealed a concentration-related increase in subcutaneous fibromas and fibrosarcomas (Table 5-3). The increases over control rates in high concentration females with fibrosarcomas or total connective tissue

TABLE 5-3

Incidence of Subcutaneous Tumors in B6C3F1 Female Mice Administered Dietary Concentrations of Technical Grade PCNB for 80 Weeks[a,b]

Concentration (ppm)	Tumor Type	Tumor Incidence (p value)
0	fibroma	0/90
100	fibroma	1/95
400	fibroma	1/91
1200	fibroma	1/91
0	fibrosarcoma	0/90 (1.0×10^{-5})[c]
100	fibrosarcoma	2/95
400	fibrosarcoma	2/91
1200	fibrosarcoma	11/91 (3.8×10^{-4})[d]
0	combined fibroma/ fibrosarcoma	0/90 (2.0×10^{-5})[c]
100		3/95
400		3/91
1200		12/91 (1.8×10^{-4})[d]

QUALITY OF EVIDENCE

Strengths of Study: Large number of mice in all groups. Concentration-related increases in fibrosarcoma incidences. Relevant route of exposure for a sufficiently long period of time. Large number of mice/group. Three dietary concentrations. Comprehensive gross necropsy on relevant target sites.

Weakness of Study: Composition of technical-grade material unclear. Limited histopathology done. Effects of impurities (esp. HCB) unclear. Incomplete histopathology on some tissue sites.

Overall Adequate: Adequate.

[a]Source: Van der Heijden, 1974

[b]Initial number of mice/group: 100

[c]Cochran-Armitage test for dose-related trend performed at SRC

[d]Fisher Exact test performed at SRC

tumors were statistically significant ($p<0.001$) at 1200 ppm. The Cochran-Armitage test for dose-related trend was also statistically significant ($p<0.001$) in female mice for the incidences of both fibrosarcomas and fibromas/fibrosarcomas. Albert (1977) stated that tumor morphology indicated malignancy and that some tumors had metastasized to the liver and lungs. In male mice, there was evidence suggestive of a concentration-related increase in liver nodules. This subtle trend was more prevalent for hyperplastic nodules (Section 5.5.) than for hepatomas (defined as lesions ranging from circumscribed nodules to distinct hepatic carcinomas). The incidences of lymphomas, leukemias and other tumors were not treatment-related.

Groups of 50 male and 50 female Wistar rats were administered 0, 100, 400 or 1200 ppm PCNB for 104 weeks (Anonymous, n.d.). Gross necropsies were done on all rats that died, were killed when moribund or were killed at the end of the study unless precluded by autolysis. Comprehensive histological examination of 20 rats of the control and 20 rats from the 1200 ppm group was conducted. Histological examination of all available rats in the 100 and 400 ppm groups was restricted to the liver and grossly visible lesions.

The mortality rate of 100 ppm males was higher than the control rate. All other groups of rats died at approximately the same rate as their respective controls. The most common neoplasms observed histologically were lymphoreticular tumors from peribronchial lymphoid tissue, hematopoietic neoplasia and mammary tumors. Male rats at all treatment levels had elevated incidences of reticulum cell sarcoma than controls (8/44 in controls vs. 20/47, 19/47 and 14/49 in those exposed to 100, 400, or 1200 ppm, respectively). The incidences at 100 and 400 ppm were significantly higher than control ($p=0.01$, Fisher Exact test performed at SRC), but the

incidence at 1200 ppm was not. At 100 ppm, 6/47 male rats had monocytic leukemia, relative to 6/44 controls; however, the incidence of this blood dyscrasia was not concentration-related. PCNB treatment also did not greatly affect the number of female rats with either mammary adenomas or adenocarcinomas (3/48 in controls vs. 5/47, 7/43, and 4/44 in rats treated with 100, 400 and 1200 ppm, respectively). The observed hepatocellular damage in rats was hyperplastic rather than malignant in nature; therefore, there were no significant treatment-related increased incidences of liver tumors in the rats.

Groups of 10 male and 10 female outbred albino mice were treated with 0.2 mℓ PCNB in 0.3% acetone, starting at 6-8 weeks of age (Searle, 1966). The compound was painted onto the shaved backs of the mice twice weekly for 12 weeks, followed by treatment with croton oil for 20 weeks. Surviving mice were untreated for a subsequent 20 weeks, after which they were sacrificed. Throughout the 40 weeks, there were marked increases in both skin papillomas and total tumors in the treated mice compared with acetone controls. These effects were more prominent in the females.

A BRL (1968a) study involved a single, subcutaneous injection of 1000 mg/kg PCNB in gelatin into the napes of 28-day-old male and female B6C3F1 and B6AKF1 mice. Eighteen mice of each sex were used. The mice were then observed for 18 months, after which survivors were sacrificed for gross and microscopic analysis. Six groups of negative controls, a vehicle control receiving gelatin on day 28, and seven positive controls were also studied. Of a total of 11 sites and tumor types assessed, the single treatment had no effect on increasing neoplastic incidence.

5.2. MUTAGENICITY

Papers regarding the genotoxicity of PCNB and details regarding their experimental design are shown in Table 5-4. PCNB was negative for reverse mutation in Salmonella typhimurium strains (Waters et al., 1982; Moriya et al., 1983; Haworth et al., 1983) and in Escherichia coli WP2 repair-proficient strains (Shirasu et al., 1976; Waters et al., 1982; Moriya et al., 1983) with and without metabolic activation. Clarke (1971), however, reported positive results for reverse mutation in a WP2 repair-deficient strain. Results were negative for S. typhimurium and Serratia marcescens host-mediated assays for reverse mutations (Buselmaier et al., 1972), for forward mutations to galactose prototrophy in E. Coli strain 343 (Mohn, 1971) and in a Bacillus subtilis rec assay (Shirasu et al., 1976). In eukaryotic systems, PCNB gave negative results for mitotic recombinations in Saccharomyces cerevisiae, for unscheduled DNA synthesis in a human fibroblast culture (Waters et al., 1982) and for sex-linked recessive lethals in Drosophila (Vogel and Chandler, 1974; Waters et al., 1982). PCNB was negative for dominant lethal mutations when administered in the diet to male mice (Jorgenson et al., 1976).

5.3. TERATOGENICITY

In a 3-generation study in CD rats, Borzelleca et al. (1971) administered dietary levels of 0, 5, 50 or 500 ppm 97.8% PCNB to 20 males and 20 females for 11 weeks. After this time, breeding pairs were established, and the females allowed to raise two separate sets of litters (designated F_{1a} and F_{1b}). From each group, 25 males and 25 females were maintained on the same diet until 105 days of age, at which time they were bred to produce F_{2a} and F_{2b} litters. The F_{2b} generation was maintained on the same

TABLE 5-4

Mutagenicity Testing of PCNB

Assay	Indicator/Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium strains TA98, 100, 1535, 1537, 1538	NS	plate incorporation	minimum of 6 (unspecified) concentrations	±S-9	-	Response defined as lack of dose-related increase in revertants in any strain	Waters et al., 1982
Reverse mutation	S. typhimurium strains TA98, 100, 1535, 1537, 1538	NS	plate incorporation	up to 5000 µg/plate	±S-9	=	NC	Moriya et al., 1983
Reverse mutation	S. typhimurium strains TA98, 100, 1535, 1537	suspended in DMSO	plate incorporation	0, 100, 333.3, 1000, 3333.3, 6666.7, or 10,000 µg/plate	±S-9	=	NC	Haworth et al., 1983
Reverse mutation	Escherichia coli strains WP2 and WP2 trv hcr	NS	spot test	NS	NS	-	NC	Shirasu et al., 1976
Reverse mutation	E. coli strains WP2 and WP2 trv hcr	NS	plate incorporation	minimum of 6 (unspecified) concentrations	±S-9	-	NC	Waters et al., 1982
Reverse mutation	E. coli strains WP2 and WP2 trv hcr	NS	plate incorporation	up to 5000 µg/plate	±S-9	=	NC	Moria et al., 1983
Reverse mutation	E. coli strains WP2 hcr$^+$ and WP2 hcr$^-$	NS	plate incorporation	NS	NS	+	A 10-fold increase in revertants in the hcr$^-$ strain only	Clarke, 1971
Host-mediated assay	S. typhimurium strain G46, Serratia marcescean strain a 21	NS	intraperito-neal injection of mice	500 mg/kg i.p.	NA	-	NC	Buselmaier et al., 1972
Forward mutation	E. coli strain 343	NS	plate incorporation	2 mg/mℓ	NS	-	NC	Mohn, 1971

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TABLE 5-4 (cont.)

Assay	Indicator/Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Rec assay	Bacillus subtilis strains H17 Rec+ and M45 Rec-	NS	spot test	NS	NS	-	NC	Shirasu et al., 1976
Mitotic recombination	Saccharomyces cerevisiae D3	NS	plate tests	5 (unspecified) concentrations	±S-9	=	NC	Waters et al., 1982
Sex-linked recessive	Drosophila melanogaster	NS	males in vivo	NS	NA	-	NC	Waters et al., 1982
Sex-linked recessive	D. melanogaster	tested as suspension (DMSO)	males in vivo	3.3 mM	NA	-	NC	Vogel and Chandler, 1974
Unscheduled DNA synthesis	Human lung fibroblasts strain WI-38	NS	liquid culture	5 (unspecified) concentrations	±S-9	=	NC	Waters et al., 1982
Dominant lethal	mice	NS	diet for 7 weeks	three dose levels (not specified)	NA	-	NC	Jorgenson et al., 1976

NS = Not specified; NA = not available

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protocol to produce F_{3a} and F_{3b} litters. There were no treatment-related effects, in any generation, on embryolethality or structural defects. Additionally, histopathological analysis of treated F_{3b} pups at 2 months of age revealed no evidence of microscopic anomalies.

Administration of PCNB in corn oil by gavage at 0, 50, 100 or 200 mg/kg/day to groups of 20 Wistar rats on days 6-15 of gestation did not result in increased incidences of malformation or skeletal variations of the offspring (Khera and Villeneuve, 1975). There was no effect on fetal weight; however, at 200 mg/kg there was reduced mean maternal body weight and decreased number of live fetuses/litter. Jordan et al. (1975) found no effects on fertility, numbers of implantations/corpora lutea, resorptions, fetal weight, fetal sex ratio, fetal viability and no increased and no increased incidences of malformations or skeletal variations in CD rats treated with PCNB in corn oil by gavage at $\leq$125 mg/kg/day on days 6-15 of gestation.

BRL (1968b) conducted a reproductive study of PCNB in C57B16 and AKR strains of mice. The PCNB used was reported to be contaminated with 11% HCB. PCNB in honey was administered by gavage to nine AKR dams at 464 mg/kg/day on days 6-15 of gestation, then sacrificed on day 19. This treatment did not increase the incidence of terata in AKR fetuses relative to untreated and vehicle-treated controls; however, fetuses from 8-12 C57B16 dams administered 215 or 464 mg/kg PCNB showed incidences of terata (24 and 26%, respectively) significantly greater than the high spontaneous rates observed in the honey-treated controls (10%). Most striking were the elevated incidences of renal agenesis (usually unilateral) in fetuses of 9/10 C57B16 dams receiving 464 mg/kg on gestation days 6-15 (14/56) or 6-10 (9/56) but not days 10-15 (0/51). The incidence of renal agenesis in

controls was 0/164. An increased occurrence of microphthalmia was also observed in the offspring of C57BL6 dams administered 464 mg/kg PCNB from days 6-10 (19/58) compared with 14/164 controls. There were no consistent treatment-related effects on maternal body weight or liver weight.

In an attempt to replicate the BRL (1968b) study, Courtney et al. (1976) administered PCNB, contaminated with 11% HCB, to 23 C57B16 mouse dams. The mice received 500 mg/kg PCNB in corn oil on each of days 7-11 of gestation. Treatment significantly ($p<0.001$) elevated the average number of abnormal fetuses/litter. The most prominent aberrations were cleft palate, anophthalmia, microphthalmia and, in particular, renal agenesis. No effect on maternal body weight was observed. When gravid CD rats were dosed with 500 mg/kg HCB-contaminated PCNB or 99% (analytical) PCNB, from days 7-18 of gestation, there was no similar increase in fetal aberrations.

These results led Courtney et al. (1976) to question whether the teratogenic effect observed in mice was caused by PCNB or the contaminant HCB. Female CD-1 mice were subjected to daily gavage with PCNB of various purities, as well as HCB and other contaminants, from gestational days 7-16. Administration of 500 mg/kg/day "pure" (<20 ppm HCB) PCNB led to significantly increased fetal mortality and incidences of abnormal fetuses/litter ($p<0.05$) compared with controls. This dose also increased the incidence of cleft palate (7.9 treated vs. 0% in controls) and greatly increased the incidence of clubfoot (23.1% of treated vs. 5.1% of control). No renal damage in fetuses was observed. There was no effect on maternal body weight or liver weight. At 500 mg/kg/day (contaminated) PCNB, the number of abnormal fetuses/litter was greatly increased ($p<0.01$). This effect was attributable solely to an increase in the percentage of cleft palates/litter (26% in treated fetuses vs. 0% in controls). Dams receiving contaminated

PCNB had significantly increased relative liver weights. Finally, in fetuses of mice given 100 mg/kg/day of HCB (a dose ~2 times that ingested by the mice receiving contaminated PCNB), there was also a significant ($p<0.05$) increase in the number of abnormal fetuses/litter. In this instance, however, the aberrations were primarily small kidneys, enlarged renal pelvis and renal agenesis. These results led the investigators to conclude that HCB was the teratogenic agent involved in causing kidney terata. Courtney et al. (1976) stated that the high incidence of cleft palates reflected two fetuses with cleft palate in each of two litters that had a high mortality, which made the overall incidence appear higher than it actually may have been.

Fytizias-Danielidou (1975) administered 75% PCNB in methylcellulose (0 or 400 mg/kg/day) to Wistar rats on days 7-10 or 12-15 of gestation. There was no effect on percent resorptions, percent live fetuses and no malformations. Fetal body weight was appreciably decreased compared with controls.

5.4. OTHER REPRODUCTIVE EFFECTS

In the 3-generation study conducted by Borzelleca et al. (1971) (see Section 5.3.), each generation of CD rats was fed 0, 5, 50 or 500 ppm 97.8% PCNB. Each generation of breeding females was allowed to have two litters. For the six series of matings, there were no treatment-related effects on fertility, gestation time, pup viability, lactation, number of pups born or weaned, weanling weights or number of stillborn.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

Borzelleca et al. (1971) gave groups of four male and four female purebred beagle dogs (4.5 months old) dietary levels of 0, 5, 30, 180 or 1080

ppm 97.8% PCNB in ground dog meal for 2 years. Food consumption was measured daily and body weights weekly. Hematological studies and urinalyses were performed on all dogs at 0, 3, 6, 12, 18 and 24 months. Blood biochemistries and liver function tests were performed on the control and high-dose dogs at the same intervals, and those dogs exposed to the intermediate concentrations were tested both before the start of administration and immediately after sacrifice. External lymph nodes were palpated weekly and the females were observed for any abnormalities in their estrous cycle. One dog/sex was sacrificed after 1 year for histopathological analysis of 22 tissues, and the remaining dogs were similarly examined after the 2-year period. Heart, spleen, liver, kidneys and testes weights were recorded.

There were no treatment-related effects on body weights, food consumption, estrous cycle, urinalysis or lymph node size. Although males fed the 30 and 180 ppm diets had lower hematocrit values at 18 months than did the control males, this result was not found in the males fed 1080 ppm. At 3 months, there were statistically significant, but slight, increases in SGOT levels in high-dose females. In combined data for both sexes given 1080 ppm, there were also slight increases in SAP levels at 12 months, which were not observed at 18 or 24 months. The investigators stated that relative hepatic weights in the 1080 ppm dogs were scarcely elevated over those of controls. The only significant histopathological change was a dose-related increased severity of cholestatic hepatosis with secondary bile nephrosis in dogs given 108 and 1080 ppm, but Borzelleca et al. (1971) considered these lesions reversible.

Albert (1977) reviewed 80-week dietary studies involving technical grade PCNB administration in Swiss albino mice of both sexes. The primary report

has been cited by U.S. EPA (1977) as Van der Heijden and Til (1974), which was unavailable in the published literature. PCNB was reported to be 98.2% pure. Dietary levels in 100 mice/sex/group were 0, 100, 400 and 1200 ppm. High-dose males and females had decreased body weight gain; the decrease was significant (p=0.01) in males compared with controls. In all treated mice, decreased lymphocytes and increased neutrophils, relative to controls, was observed at 75 weeks. Starting at 400 ppm, the relative liver weights of treated mice were significantly (p=0.01 or 0.001) above control weights, and the females given 1200 ppm had elevated (p=0.001) relative kidney weights. In addition to hepatomas (see Section 5.1.), male mice showed a treatment-related increase in hyperplastic liver nodules, which was slightly above control values even at the 100 ppm level. The investigators described these lesions as "areas of hepatocytes with basophilic cytoplasmic changes and hyperplastic nodules mainly characterized by normal appearance of cells and architecture." There was no mention of similar hepatic damage in females.

Albert (1977) also reviewed an unpublished study by Anonymous (n.d.) in which groups of 50 male and 50 female Wistar rats were given dietary PCNB at 0, 100, 400 or 1200 ppm for 104 weeks. The rats were weighed and food consumption was monitored, and hematologies were performed at weeks 52 and 104. Blood biochemistry measures were taken at the end of the study and gross necropsies performed at terminal sacrifice. Detailed microscopic examination of the tissues, especially the liver, was performed on selected samples. Detailed histological examination was restricted to only 20 males and 20 females in each of the control and 1200 ppm groups. All other necropsies were restricted to the liver or to tissues suspected of being tumorous.

At 400 ppm, there were statistically significant, but unspecified, differences from controls in packed cell volume and blood cell counts that were due to treatment with the intermediate doses. SGPT levels were lower in all treated groups and serum protein levels were reported lower in males given 400 or 1200 ppm. At 1200 ppm, relative liver ($p<0.001$) and kidney ($p<0.05$) weights were increased in both sexes. Rats exposed to $\geq$100 ppm had dose-related increased severity and incidence of liver toxicity, described as enlargement of hepatocytes with an increased incidence of fine vacuolar fatty metamorphosis and isolated cell necrosis. At $\geq$400 ppm, there was frequent centrilobular hepatocytomegaly, which at advanced stages was characterized by Mallory bodies. Mortality rates in both sexes were not concentration-related.

Finnegan et al. (1958) administered 10 male and 10 female albino rats 0, 25, 100, 300, 1000 or 2500 ppm technical grade PCNB for 2 years. The compound was provided as a commercial powder containing 20% PCNB, 77% Pyrak ABB (a pyrophyllite carrier) and 3% Armour "Sticker." Hawley (1981) defines pyrophyllite as a hydrous aluminum silicate of low toxicity. Effects of this substance, or of Sticker, on the gastrointestinal absorption of PCNB are unclear. The rats were weighed weekly and hematological examinations were performed during the 11th and 24th months. Fifteen tissues from moribund rats and rats sacrificed at the end of the 2-year period were studied histologically. There were no differences in mortality, hematological values or histological parameters that were due to treatment. Males given 2500 ppm had sporadic decreases in growth rate. The growth rates in females at 100, 300 and 2500 ppm were also significantly impaired at most sampling points; however, the females given 1000 ppm gained weight at the same rate as controls. Finnegan et al. (1958) also found liver cell enlargement and

pale-staining cytoplasm in the tissues of three dogs/group exposed for 1 year to 25-1000 ppm PCNB. In the absence of control data, these results are difficult to interpret.

NCI (1978) conducted a 78-week technical-grade PCNB feeding experiment in B6C3F1 mice and Osborne-Mendel rats (see Section 5.1.). Fifty animals/sex/strain were in the experimental groups, whereas 20 animals were in each control group. The TWA concentrations for rats were 5417 ppm (low-dose males), 10,064 ppm (high-dose males), 7875 ppm (low-dose females) and 14,635 ppm (high-dose females). TWA doses for mice were 2606 ppm (low-dose males), 5213 ppm (high-dose males), 4093 ppm (low-dose females) and 8187 ppm (high-dose females). Mice were sacrificed 14-15 weeks after treatment and rats were sacrificed at 33-35 weeks after treatment. Gross and comprehensive histological examination were performed. NCI (1978) noted abdominal urine staining and a "hunched" appearance principally in the high-dose rats. These effects persisted in the treated rats despite reductions in food concentrations after 13 and 52 weeks. These signs, however, occurred equally among control and experimental rats after treatment ended on week 78. By week 34, 27% of the low-dose male mice and 70% of the high-dose males had a hunched appearance, which persisted until sacrifice. Both sexes of rats showed a concentration-related inhibition of body weight gain throughout treatment. This effect was also apparent in female mice after the 35th treatment week. In neither species was there any manifestation of non-neoplastic tissue pathology that was due to PCNB administration.

In a repeat bioassay in B6C3F1 mice, NTP (1986) administered PCNB (>99% PCNB; 0.07±0.01% HCB) to groups of 50 males and 50 females at dietary levels of 0, 2500 or 5000 ppm for 103 weeks (see Section 5.1.). These dietary levels provided doses of PCNB of 0, 400 and 1000 mg/kg/day to males and 0,

600 and 1400 mg/kg/day to females as calculated by NTP (1986) from food consumption data. There was no effect on body weight or mortality and no compound-related histological lesions in male mice. In female mice, there was a dose-related decreased body weight gain and survival. The survival of the high-dose females was significantly lower than controls after week 86 ($p=0.005$). The reduced survival was attributed to a combination of PCNB toxicity and ovarian abscesses. The ovarian abscesses occurred in 12/49 controls, 22/50 low-dose ($p=0.03$) and 29/50 high-dose ($p<0.001$) females and were attributed to Klebsiella infection. Dose-related increased incidences of hematopoiesis in the liver and spleen and plasma cell hyperplasia of the mediastinal lymph nodes were also observed in treated females, but these lesions were considered to be secondary to bacterial infection. There was a significant ($p\leq0.05$) correlation between these lesions and the presence of ovarian abscesses in the same mouse. Because the incidence of ovarian abscess was greater in the PCNB-treated groups and was dose-related, NTP (1986) suggested that PCNB predisposed the female mice to infection.

Groups of 20 Wistar rats/sex were administered, by intragastric intubation, 0, 100, 200 or 400 mg/kg PCNB, 6 days/week for up to 8 months (Fytizias-Danielidou, 1975). The pesticide was given as a 75% pure suspension in methylcellulose; composition of the non-PCNB fraction was not stated. The rats were weighed weekly and placed in metabolism cages for urine volume measurements. At 8-week intervals, blood samples for PCB and WBC counts were taken. Also every 8 weeks, four rats/group were sacrificed for histological examination.

High-dose administration was lethal to 30% of the males and 20% of the females, whereas 200 mg/kg was fatal to 10% of the exposed females. There

were no other treatment-related deaths in any group. At 200 and 400 mg/kg, the rats showed inhibitions of weight gain relative to controls. These effects were transient and had reversed by 6 months. Treatment at the two highest doses caused a polyuria, which was pronounced at the 400 mg/kg dose. After 6 months, lesions and cellular necrosis in all groups were observed in the periportal regions of the livers of treated rats; at 8 months, the livers showed regeneration. Mean relative liver weights were elevated in rats treated with 200 or 400 ppm at all sacrifices. Degeneration of the tubular epithelium, and occasionally the collecting ducts, were the most common exposure-related renal lesions. Hematological values were unaffected by treatment.

Koegel et al. (1979b) administered >99.9% ^{14}C-PCNB (specific activity = 0.8 mC/mM) to two male and two female rhesus monkeys on sucrose pellets for 70 days. The amount administered corresponded to 2 ppm of the daily diet. One male and one female were sacrificed 1 day after the last dose for complete necropsy. On days 0, 19, 48 and 76, blood samples were taken from the two remaining monkeys for clinical, chemical and hematological analyses. There were no clear-cut dose-related effects of ^{14}C-PCNB treatment on hemoglobin content, packed cell volume or RBC or WBC counts. Moreover, levels of serum electrolytes, enzymes (SGOT, SGPT and LDH), creatinine, BUN and bilirubin remained within normal limits at all time points sampled. ^{14}C-PCNB administration also did not affect circulating cortisol, lutenizing hormone, follicle stimulating hormone or progesterone levels, and had no noticeable influence on tissue morphology. Results on highly variable measures, such as serum LDH and bilirubin levels, were difficult to assess because of the small sample sizes.

In an oral study of subchronic exposure, Finnegan et al. (1958) gave groups of five albino rats of each sex technical grade PCNB at dietary concentrations of 0, 63.5, 635, 1250, 2500 or 5000 ppm for 13 weeks. PCNB was supplied as a 20% dust. The highest concentration was so debilitating that on week 3 all surviving 5000 ppm rats were sacrificed. Growth was significantly ($p<0.05$) inhibited only at 2500 ppm in the males, whereas female growth rates were unaffected by treatment. PCNB administration at 2500 ppm also failed to affect survival rates or hematological results. All groups except the 63.5 ppm females had elevated relative liver weights, and males at 1250 and 2500 ppm showed elevated kidney weight ratios. Examination of 13 tissues taken from sacrificed rats of all dose groups revealed only fine hepatocellular cytoplasmic vacuolation in the rats given 5000 ppm.

In establishing the MTD of 97% PCNB for chronic oral administration, the NCI (1978) conducted 6-week dietary studies using groups of five male and five female B6C3F1 mice and Osborne-Mendel rats. Dietary concentrations were 0, 2150, 4640, 10,000, 21,500 and 46,400 ppm PCNB. Following the 6-week treatment period, surviving animals were observed for 2 weeks before sacrifice. All male rats and one female rat died at the high concentration. At 21,500 ppm, mean body weight depressions were 42 and 16% in males and females, respectively. In mice, 4/5 males receiving 4640 ppm, and 2/5 females receiving 10,000 ppm, died. At 2150 ppm (males) and 4640 ppm (females), body weight depression compared with controls was $\leq$10%.

In establishing the MTD of 99% PCNB for chronic oral administration, NTP (1986) conducted a 13-week dietary study using groups of 10 male and 10 female B6C3F1 mice. Males received diets containing 0, 1250, 2500, 5000, 10,000 or 20,000 ppm PCNB, and females received diets containing 0, 2500, 5000, 10,000, 20,000 or 40,000 ppm. All 40,000 ppm females died during the study and 8/9 had lymphoid depletion of the spleen, lymph nodes or thymus.

Females at 20,000 and 40,000 ppm and males at 20,000 ppm were emaciated. Relative liver weights were significantly increased in males at $\geq$2500 ppm and females at $\geq$5000 ppm.

A 3-week dietary exposure to 2000 ppm PCNB increased relative liver weight and induced hepatic microsomal oxidation in female Wistar-JCL rats (Kawano et al., 1979). This treatment also significantly decreased SGPT ($p<0.01$), but increased serum carbohydrate and cholinesterase ($p<0.05$) levels, relative to controls.

5.6. OTHER RELEVANT INFORMATION

A human case of kerato conjunctivitis that was due to PCNB exposure from a pesticide spray was described in a Japanese abstract (Fujita et al., 1976). Corneal injury was seen early in exposure and remission was slow. No further details were provided.

PCNB has proven to be only slightly acutely toxic when given orally. LD_{50} values after single-dose administration in rats (Finnegan et al., 1958; Borzelleca et al., 1971; Renner, 1980) and voles (Cholakis et al., 1981) ranged from 1.65 to >12 g/kg. Finnegan et al. (1958) noted that technical grade PCNB was more acutely toxic than a wettable powder containing 75% of the fungicide, but provided no further details concerning the composition of each sample. Cholakis et al. (1981) found 30-day LC_{50}s of 11,290-42,840 ppm for voles given dietary analytical PCNB. At doses just below those that are acutely lethal, specific clinical signs and other manifestations of toxicity are often difficult to discern. A female rhesus monkey given a 91 mg/kg dose of >99.9% pure ^{14}C-PCNB had a transient methemoglobinemia only on the first day after oral dosing (Koegel et al., 1979b). Analysis of blood samples, drawn through 16 days postadministration, did not indicate any changes in hemoglobin content, packed cell volume or RBC and WBC counts. Schumann and Borzelleca (1978) orally administered

technical grade PCNB at 0 (corn oil control), 1600 or 2400 mg/kg to groups of four cats. Both noncontrol doses elevated methemoglobinemia and Heinz body formation 24, 48 and 72 hours after administration, but the effect of 1600 mg/kg was most significant. Schumann and Borzelleca (1978) attributed this finding to the possibility that, at the high dose, precipitated PCNB in corn oil may be only slowly absorbed through the gut mucosa. Although these doses were far greater than the one used by Koegel et al. (1979b), differences in results may also have been due to the sensitivity of cats to methemoglobinemia formation (Schumann and Borzelleca, 1978).

5.7. SUMMARY

There have been five chronic bioassays involving dietary administration of PCNB. BRL (1968a) administered a TWA concentration of 1298 ppm PCNB to 18 male and 18 female B6C3F1 and B6AKF1 mice for 77 weeks. Courtney et al. (1976) later confirmed that the sample was contaminated with 11% HCB. The only positive neoplastic results were increased incidences of hepatomas in female B6C3F1 and male B6AKF1 mice ($p<0.001$), compared with negative controls. Cabral et al. (1977, 1978), however, noted increased incidences of this tumor type in hamsters treated with 50-200 ppm and mice treated with $\geq$100 ppm HCB alone in the diet.

NCI (1978) administered 97% PCNB to 50 male and 50 female Osborne-Mendel rats and B6C3F1 mice for 78 weeks, at two dietary levels. TWA concentrations for the low-dose rats were 5417 ppm (males) and 7875 (females) ppm, and for the high-dose rats were 10,064 ppm (males) and 14,635 ppm (females). TWA concentrations for the low-dose mice were 2606 ppm (males) and 4093 ppm (females), and for the high-dose mice were 5213 ppm (males) and 8187 ppm (females). Compared with control groups of 20 rats each, the treated rats showed no significant increases in tumor incidence. Among the mice, low-dose males and high-dose females had statistically insignificant (by the

Fisher exact test) elevations in hepatocellular carcinomas, over control rats. These results were especially difficult to evaluate because of the small number of male mice that survived to terminal sacrifice and the large number of tissue sites that were autolyzed in female mice. In a repeat study by NTP (1986) that used highly purified PCNB (>99% PCNB, 0.07±0.01% HCB), B6C3F1 mice were given the compound in the diet at 2500 or 5000 ppm for 103 weeks. No evidence of carcinogenicity was found, although high mortality in the treated female mice decreased the sensitivity of the study.

Two oral chronic bioassays using technical grade PCNB were performed in Swiss mice (Van der Heijden and Til, 1974) and albino rats (Anonymous, n.d.). The compound was administered for 80 weeks (mice) and 104 weeks (rats), at 0, 100, 400 or 1200 ppm. In high-dose female mice there were increased ($p<0.001$) incidences of fibrosarcomas and combined fibromas/fibrosarcomas ($p<0.001$), relative to control values. Cochran-Armitage tests were also statistically significant ($p<0.001$) in female mice for these tumor types. In male mice, there was suggestive evidence of a treatment-related increase in liver nodules, which was probably a hyperplastic rather than a neoplastic effect. In rats of either sex, there appeared to be no treatment-related increase in tumorigenesis.

An analysis of tumor responses reported for PCNB contaminated with HCB (BRL, 1986a; Courtney et al., 1976; NCI, 1978) shows that there is a numerical relationship between the HCB contamination level and the tumor responses. In view of all the data, it is possible that the HCB contamination is influential in the carcinogenic activity of PCNB.

PCNB has been found to initiate carcinogenic activity in initiation-promotion studies (Searle, 1966), but not when given as a single subcutaneous injection to B6C3F1 or B6AKF1 mice (BRL, 1968b). In vitro, PCNB was

not mutagenic in reverse mutation assays using S. typhimurium (Waters et al., 1982; Haworth et al., 1983) or E. coli (Shirasu et al., 1976; Waters et al., 1982; Moriya et al., 1983) with or without metabolic activation. It did cause reverse mutations in an E. coli WP-2 repair-deficient strain (Clarke, 1971). In an assay for forward mutations in E. coli (Mohn, 1971), the rec assay in Bacillus subtilis (Shirasu et al., 1976) and mitotic recombination in Saccharomyces cerevisiae (Waters et al., 1982), PCNB was not genotoxic. The compound also had no effects in sex-linked recessive lethal assays in Drosophila melanogaster (Waters et al., 1982; Vogel and Chandler, 1974), a dominant lethal assay in mice (Jorgenson et al., 1976), unscheduled DNA synthesis in human fibroblasts (Waters et al., 1982) or in host-mediated assays in S. typhimurium and Serratia marcescens (Buselmaier et al., 1972).

In a 3-generation study using CD rats, there were no effects on reproductive indices or terata in any generation that were due to the continual administration of up to 500 ppm PCNB in the diet (Borzelleca et al., 1971). No microscopic anomalies were found in the tissues of treated third-generation weanlings. Oral treatment of rats with 200 mg/kg PCNB on gestation days 6-15 led to losses in maternal body weight gain and decreased numbers of liveborn fetuses/litter (Khera and Villeneuve, 1975). There were no dose-related effects on the appearance of terata or postnatal fetal development at $\leq$125 mg/kg (Jordan et al., 1975). In C57B16 (but not AKR) mice, oral administration of 215 or 464 mg/kg PCNB (contaminated with 11% HCB) on days 6-15 of gestation increased the incidences of renal agenesis and, to a lesser extent, microphthalmia, in fetuses (BRL, 1968b). The effects on kidney development were also observed when administration was on gestation days 6-10, but not on days 10-15.

Courtney et al. (1976) conducted a series of studies that were designed to pinpoint the causative factors of the reported renal teratogenicity. Administration of 500 mg/kg PCNB contaminated with 11% HCB to C57B16 mice on gestation days 7-11 increased the incidence of renal and eye malformations and cleft palates in exposed fetuses. There were no teratogenic effects in pups of CD rats dosed with 500 mg/kg contaminated PCNB on a longer treatment schedule. In CD-1 mice dosed daily throughout organogenesis with either 500 mg/kg contaminated PCNB or 100 mg/kg HCB, there were statistically significant increases in the incidences of cleft palate and renal malformations, respectively. Dosing with 500 mg/kg pure PCNB increased the incidence of clubfoot and fetal mortality. This regimen did not, however, provoke renal agenesis in the offspring and only slightly increased the incidence of cleft palate. The authors concluded that HCB was the etiological agent in renal and ophthalmological terata.

Two-year administration of 0, 5, 30, 180 or 1080 ppm 97.8% PCNB to three male and three female beagles/group, in the diet, had no consistent effect on the body weights, clinical signs, urinalysis results or blood biochemistries (Borzelleca et al., 1971). At the high concentration, the investigators found slightly elevated relative hepatic weights in the high-dose dogs and cholestatic hepatosis and secondary bile nephrosis at 108 and 1080 ppm.

In a long-term study, Swiss mice were exposed to 0, 100, 400 or 1200 ppm PCNB (Van der Heijden and Til, 1974). After 80 weeks, males showed a treatment-related increase in hyperplastic hepatic nodules that was slightly elevated at 100 ppm but markedly increased at 400 and 1200 ppm; this effect was accompanied by hepatomegaly at 400 and 1200 ppm. The appearance of

hepatic nodules was not observed in females, although high-dose females were shown to have increased kidney weights. In another study, Wistar rats were given dietary PCNB at 0, 100, 400 or 1200 ppm for 104 weeks (Anonymous, n.d.). At 1200 ppm, relative kidney and liver weights were higher than control values. In both sexes, indications of hepatotoxicity, including enlarged centrilobular hepatocytes, fatty centrilobular metamorphosis and single cell necrosis were slightly elevated at the 100 ppm level and markedly elevated at the higher concentrations. Female albino rats given technical grade PCNB as a 20% powder for 2 years, up to dietary levels of 23,500 ppm, had sporadic inhibition in growth rates (Finnegan et al., 1958). There were no treatment-related effects upon mortality rates, hematologies or incidences of tissue damage. These investigators also found hepatocellular enlargement and pale-staining cytoplasm in groups of three dogs exposed for 1 year to 25-1000 ppm PCNB in the diet. Two strains of mice given 1298 ppm "contaminated" PCNB in the diet for 77 weeks had increased incidences of pneumonia and lymphoid infiltration, relative to controls (BRL, 1968a). In a more comprehensive bioassay, the NCI (1978) found that TWA concentrations of 10,064-14,635 ppm dietary PCNB led to a "hunched" appearance and abdominal urine stains in male and female rats. At these dietary levels and at lower levels (5417-7875 ppm), there were inhibitions in weight gain relative to controls. The hunched appearance also occurred in treated mice, and female mice treated with up to 9000 ppm (TWA = 8187 ppm) PCNB showed an inhibition of weight gain. No treatment-related effects or nonneoplastic tissue pathology were noted.

In a repeat study in B6C3F1 mice, there was a dose-related decreased body weight gain, increased mortality and increased incidence of ovarian abscesses in females given 2500 and 5000 ppm in the diet for 103 weeks (NTP,

1986). The increased mortality was attributed to a combination of the toxicity of PCNB and the ovarian infections. It was suggested that the PCNB-treatment predisposed the female mice to infection.

Fytzias-Danielidou (1975) administered a 75% pure suspension of PCNB to Wistar rats by gavage, 6 days/week for up to 32 weeks. Serial sacrifices were made every 8 weeks. Exposure to 400 mg/kg (and to a lesser extent 200 mg/kg) led to increases in mortality, transient inhibitions in weight gain, polyuria, hepatocellular necrosis, hepatomegaly and degeneration of the renal tubular epithelium. There were no changes in hematological parameters.

A number of animal studies involving subchronic oral exposure to PCNB have been conducted. Koegel et al. (1979b) found no effects of 2 ppm dietary PCNB, given over 70-80 days, on four rhesus monkeys, relative to historic controls. In 13-week oral dosing, Finnegan et al. (1958) noted mortality and morbidity in rats given a dietary concentration of 5000 ppm PCNB (as a 20% dust), and inhibition in growth rate in males given 2500 ppm. At lower concentrations, there were increases in relative liver and kidney weights. The NCI (1978) observed body weight gain depressions and mortality in rats exposed to $\geq$21,500 ppm or greater PCNB, and in mice exposed to 4640 (males) or 10,000 (females) ppm, for a 6-week period. NTP (1986) found significantly increased relative liver weights in male mice treated at $\geq$2500 ppm and females at $\geq$5000 ppm PCNB in the diet for 13 weeks. Mice of both sexes were emaciated at $\geq$20,000 ppm. The 40,000 ppm level was fatal to female mice and resulted in lymphoid depletion. Finally, Kawano et al. (1979) noted evidence of increased hepatic microsomal activity in rats given 2000 ppm PCNB, of an unknown purity, for 3 weeks.

Acute oral LD_{50} values for PCNB in rodents range from 1.65 to >12 g/kg. The technical grade material has been found to be more hazardous than the wettable powder in the same concentration basis (Finnegan et al., 1958; Borzelleca et al., 1971; Renner, 1980; Cholakis et al., 1981). Schumann and Borzelleca (1978) reported methemoglobinemia in rats after oral administration of PCNB, an effect not observed by Koegel et al. (1979b) in rhesus monkeys. Dose and species differences were the two most likely reasons for these discrepancies.

6. AQUATIC TOXICITY

6.1. ACUTE

Little pertinent information is available regarding the toxicity of PCNB to aquatic organisms in the available literature. Hasimoto and Nishiuchi (1981) found that PCNB toxicity to carp (species not specified) increased at higher temperatures. They reported the following 24-hour LC_{50} values: 15°, 40 mg/ℓ; 20°, 20 mg/ℓ; 25°, 15 mg/ℓ; 30°, 3.8 mg/ℓ and 35°, 1 mg/ℓ. Kuroda (1975) found that exposure to 75 mg/ℓ for 48 hours resulted in some mortality of pink salmon, Oncorhynchus gorbuscha. Kanazawa (1981) reported that the 48-hour LC_{50} for carp was >10 mg/ℓ and the 3-hour LC_{50} for Daphnia sp. was >40 mg/ℓ. The ciliate protozoan, Colpidium campylum, was unaffected by 10 mg/ℓ Quintozen (Dive et al., 1980), but a 0.001% dilution of Terrafun 75 (75% PCNB) was lethal to Paramecium aureliu (Komala, 1976).

6.2. CHRONIC

Pertinent data regarding chronic toxicity of PCNB to aquatic organisms could not be located in the available literature as cited in the Appendix.

6.3. PLANTS

Pertinent data regarding effects of PCNB on aquatic plants could not be located in the available literature as cited in the Appendix.

6.4. RESIDUES

Data concerning PCNB residues in aquatic organisms are presented in Table 6-1. There is a large variation in the degree to which PCNB is bioaccumulated by different species. Part of this variability may be due to the fact that in studies where radiolabeled PCNB was used, results may have

TABLE 6-1

Data Concerning PCNB Residues in Aquatic Organisms

Species	Water Concentration (mg/ℓ)	Duration (days)	Tissue Concentration (µg/ℓ)	BCF	Reference
			PLANTS		
Alga, Chlorella fusca	0.05	1	NR	3100	Freitag et al., 1982
	0.049	1	NR	14,000-22,000	Korte et al., 1978
Alga, Oedogonium cardiacum	0.001	20-42	0-0.002	NR	Isensee and Yockim, 1980
	0.01	11-65	0.0003-0.36	NR	Isensee and Yockim, 1980
	0.1	7-65	0.005-0.472	NR	Isensee and Yockim, 1980
Invertebrates					
Snail, Physa sp.	0.0002	NR	0.350	NR	Metcalf et al., 1979
Snail, Helisoma sp.	0.001	15-42	0-2.3	NR	Isensee and Yockim, 1980
	0.01	15-56	0-13.4	NR	Isensee and Yockim, 1980
	0.1	15-56	1.2-102	NR	Isensee and Yockim, 1980
Water flea, Daphnia magna	0.001	20-42	0-1.1	NR	Isensee and Yockim, 1980
	0.01	20-42	0.1-6.4	NR	Isensee and Yockim, 1980
	0.1	1-45	4.4-114	NR	Isensee and Yockim, 1980
Fish					
Mosquitofish Gambusia affinis	0.001	11-42	0-3.8	NR	Isensee and Yockim, 1980
	0.01	11-56	0-12.6	NR	Isensee and Yockim, 1980
	0.1	11-56	9.6-99.4	NR	Isensee and Yockim, 1980
	0.0002	NR	0.171	NR	Metcalf et al., 1979
Golden orfe, Leuciscus idus	0.0004	3	NR	1140	Korte et al., 1978
	0.006	3	NR	950	Korte et al., 1978
	0.033	2	NR	430	Korte et al., 1978
Topmouth gudgeon Pseudorasbora parva	NR	NR	NR	238	Kanazawa, 1981

NR = Not reported

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been reported as total radioactivity accumulated (i.e., PCNB and its metabolites) or as ^{14}C-PCNB alone. In general, however, it appears that PCNB and its metabolites can accumulate to a significant extent in aquatic organisms.

Bahig et al. (1981) found that PCNB was rapidly metabolized by fish, Leuciscus idus. The primary pathway was substitution of the nitro group by -SH or -OH, followed by conjugation and elimination. Dechlorination, however, did not occur to a measurable extent. Murphy et al. (1982) reported a similar mechanism in the protozoan, Tetrahymena thermophila. Isensee and Yockim (1980) also reported that PCNB was rapidly eliminated by algae, Oedogonium cardiacum, snails, Helisoma sp., Daphnia magna "crustacean" and mosquitofish, Gambusia affinis. Elimination by these species was 50-95% after 10 days in clear water.

The only quantitative monitoring data for PCNB were provided by Poels et al. (1980). They found that rainbow trout, Salmo gairdneri, exposed to Rhine River water for 6-18 months accumulated 1.3-2.5 mg/kg PCNB in adipose tissue.

6.5. SUMMARY

Little pertinent information is available concerning toxicity of PCNB to aquatic organisms in the available literature. PCNB toxicity to carp increased with increasing temperature, with 24-hour LC_{50} values ranging from 40 mg/ℓ at 15°C to 1 mg/ℓ at 35°C (Hasimoto and Nishiuchi, 1981). The lowest concentration at which toxic effects were reported for any species was 1 mg/ℓ. PCNB and its metabolites can apparently accumulate to a significant extent in fish and other aquatic biota. In the species that have been studied, PCNB metabolism involves denitrification but not dechlorination (Bahig et al., 1981; Murphy et al., 1982).

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

The U.S. EPA (1982a) lists a q_1^* of 2.62×10^{-1} $(mg/kg/day)^{-1}$ for PCNB. The q_1^* was derived by U.S. EPA (1982d) based on the incidence of hepatomas in male B6AKF1 mice treated with PCNB by gavage at 464 mg/kg/day for 3 weeks, and then in the diet at 1208 ppm for 74 weeks in the study reported by BRL (1968a) and Innes et al. (1969).

The ADI for PCNB was derived and verified by the Office of Pesticide Programs of the U.S. EPA (1986). The ADI of 0.008 mg/kg/day was based on a NOEL of 30 ppm in the diet of dogs in a study by Borzelleca and Larson (1968) converted to a dose of 0.75 mg/kg/day (food factor = 0.025) and divided by an uncertainty factor of 100. The LOAEL was 180 ppm at which liver toxicity was observed. The unpublished study by Borzelleca and Larson (1968) is the same as that published by Borzelleca et al. (1971) (see Section 5.5.).

The WHO/FAO (1974) recommended an ADI of 0.001 mg/kg/day for PCNB, based upon findings that higher doses may be teratogenic. They proposed that a more suitable ADI be based upon long-term feeding studies in at least two species. In 1976, WHO/FAO updated their analysis and presented data indicating no-effect dietary levels of 25 and 30 ppm in rats and dogs, respectively. The rat level corresponded to 1.25 mg/kg bw and the equivalent dose level for the dog was 0.75 mg/kg bw. From these data, WHO/FAO calculated an ADI of 0.007 mg/kg/day.

The U.S. EPA (1977) issued a rebuttable presumption against registration of PCNB based upon oncogenicity findings (see Section 5.1.). On April 28, 1982, the U.S. EPA (1982b) announced that the rebuttable presumption against PCNB has been terminated and made the following conclusion:

> the evidence does not positively establish a correlation between exposure to PCNB itself and oncogenicity. With regard to HCB, the available studies indicate that this impurity of PCNB is an animal carcinogen and is likely to be responsible for any potential oncogenic effect of commercial PCNB. Accordingly, ... reducing the level of HCB in PCNB should be essential to reducing any potential risks posed by PCNB products.

The Agency also listed a permanent tolerance for PCNB residues of 0.1 ppm for cottonseed, an interim tolerance of 1.0 ppm for peanuts, and interim tolerances of 0.1 ppm for bananas, beans, broccoli, brussel sprouts, cabbage, cauliflower, garlic, peppers, potatoes and tomatoes (U.S. EPA, 1982c).

7.2. AQUATIC

Guidelines and standards for PCNB for the protection of aquatic organisms could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

There have been five chronic bioassays involving dietary administration of PCNB. BRL (1968a) administered a TWA equivalent concentration of 1298 ppm PCNB to 18 male and 18 female B6C3F1 and B6AKF1 mice for 77 weeks. Courtney et al. (1976) later confirmed that the sample was contaminated with 11% HCB. The only positive neoplastic results were increased incidences of hepatomas in female B6C3F1 and male B6AKF1 mice ($p<0.001$), compared with negative controls. Cabral et al. (1977, 1978), however, noted increased incidences of this tumor type in hamsters treated with 50-200 ppm and mice treated with $\geq$100 ppm HCB alone in the diet.

NCI (1978) administered 97% PCNB (at two dietary levels) to 50 male and 50 female Osborne-Mendel rats and B6C3F1 mice for 78 weeks. TWA concentrations for the low-dose rats were 5417 ppm (males) and 7875 ppm (females), and for the high-dose rats were 10,064 ppm (males) and 14,635 ppm (females). TWA concentrations for the low-dose mice were 2606 ppm (males) and 4093 ppm (females), and for the high-dose mice were 5213 ppm (males) and 8187 ppm (females). Compared with control groups of 20 rats each, the treated rats showed no significant increases in tumor incidence. Among the mice, low-dose males and high-dose females had statistically insignificant (by the Fisher exact test) elevations (over control rats) in hepatocellular carcinomas. These results were especially difficult to evaluate because of the small number of male mice that survived to terminal sacrifice and the large number of tissue sites that were autolyzed in female mice. In a repeat study by NTP (1986) that used highly purified PCNB (>99% PCNB, 0.07$\pm$0.01% HCB), B6C3F1 mice were given the compound in the diet at 2500 or 5000 ppm for 103 weeks. No evidence of carcinogenicity was found, although high mortality in the treated female mice decreased the sensitivity of the study.

Two oral chronic bioassays using technical grade (98% pure) PCNB were conducted in Swiss mice (Van der Heijden and Til, 1974) and albino rats (Anonymous, n.d.). The compound was administered for 80 weeks (mice) and 104 weeks (rats) at 0, 100, 400 or 1200 ppm. In high-dose female mice there were increased ($p<0.001$) incidences of fibrosarcomas and combined fibromas/fibrosarcomas ($p<0.001$), relative to control values. Cochran-Armitage tests were also statistically significant ($p<0.001$) in female mice for these tumor types. In male mice, there was suggestive evidence of a treatment-related increase in liver nodules, which was probably a hyperplastic rather than a neoplastic effect. In rats of either sex, there appeared to be no treatment-related increase in tumorigenesis.

PCNB has been found to initiate carcinogenic activity in initiation-promotion studies (Searle, 1966), but not when given as a single subcutaneous injection to B6C3F1 or B6AKF1 mice (BRL, 1968b). In vitro, PCNB was not mutagenic in reverse mutation assays using S. typhimurium (Waters et al., 1982; Haworth et al., 1983) or E. coli (Shirasu et al., 1976; Waters et al., 1982; Moriya et al., 1983) with or without metabolic activation. It did cause reverse mutations in an E. coli WP-2 repair-deficient strain (Clarke, 1971). In an assay for forward mutations in E. coli (Mohn, 1971), the rec assay in B. subtilis (Shirasu et al., 1976) and mitotic recombination in S. cerevisiae (Waters et al., 1982), PCNB was not genotoxic. The compound also had no effects in sex-linked recessive lethal assays in D. melanogaster (Waters et al., 1982; Vogel and Chandler, 1974), a dominant lethal assay in mice (Jorgenson et al., 1972), unscheduled DNA synthesis in human fibroblasts (Waters et al., 1982), or in host-mediated assays in S. typhimurium and S. marcessans (Buselmaier et al., 1972).

In a 3-generation study in CD rats, there were no effects on reproductive indices or terata in any generation that were due to the continual administration of up to 500 ppm PCNB in the diet (Borzelleca et al., 1971). No microscopic anomalies were found in tissues of treated third-generation weanlings. Oral treatment of rats with 200 mg/kg PCNB for gestation days 6-15 led to losses in maternal body weight gain and decreased numbers of liveborn fetuses/litter (Khera and Villeneuve, 1975). There were no dose-related effects on the appearance of terata or postnatal fetal development at $\leq$125 mg/kg (Jordan et al., 1975). In C57BL6 but not AKR mice, oral administration of 215 or 464 mg/kg PCNB (contaminated with 11% HCB) from days 6-15 of gestation increased the incidences of renal agenesis and, to a lesser extent, microphthalmia, in fetuses (BRL, 1968b). The effects on kidney development were also observed when administration was on gestation days 6-10, but not on days 10-15.

Courtney et al. (1976) conducted a series of studies that were designed to pinpoint the causative factors of the reported renal teratogenicity. Administration of 500 mg/kg PCNB contaminated with 11% HCB to C57BL6 mice dams on gestation days 7-11 increased the incidence of renal and eye malformations and cleft palates in exposed fetuses. There were no teratogenic effects in pups of CD rats dosed with 500 mg/kg contaminated PCNB on a longer treatment schedule. In CD-1 mice dosed daily throughout organogenesis with either 500 mg/kg contaminated PCNB or 100 mg/kg HCB, there were statistically significant increased incidence of cleft palate and renal malformations, respectively. Dosing with 500 mg/kg pure PCNB increased the incidence of clubfoot and fetal mortality. This regimen did not, however, provoke renal agenesis in the offspring and only slightly increased the incidence of cleft palate. The authors concluded that HCB was the etiological agent in renal and ophthalmological terata.

Two-year administration of 0, 5, 30, 180 or 1080 ppm 97.8% PCNB to three male and three female beagles/group, in the diet, had no consistent effect on the body weights, clinical signs, urinalysis results or blood biochemistries (Borzelleca et al., 1971). At the high concentration, the investigators found slightly elevated relative hepatic weights at 1080 ppm and cholestatic hepatosis and secondary bile nephrosis at 108 and 1080 ppm.

In a long-term study, Swiss mice were exposed to 0, 100, 400 or 1200 ppm PCNB (Van der Heijden and Til, 1974). After 80 weeks, males showed a treatment-related increase in hyperplastic hepatic nodules that was slightly elevated at 100 ppm but markedly increased at 400 and 1200 ppm; this effect was accompanied by hepatomegaly at 400 and 1200 ppm. The appearance of hepatic nodules was not observed in females, although high-dose females were shown to have increased kidney weights. In another study, Wistar rats were given dietary PCNB at 0, 100, 400 or 1200 ppm for 104 weeks (Anonymous, n.d.). At 1200 ppm, relative kidney and liver weights were higher than control values. In both sexes, indications of hepatotoxicity, including enlarged centrilobular hepatocytes, fatty centrilobular metamorphosis and single cell necrosis, were slightly elevated at the 100 ppm level and markedly elevated at the higher concentrations. Female albino rats given technical grade PCNB as a 20% powder for 2 years, up to dietary levels of 23,500 ppm, had sporadic inhibition in growth rates (Finnegan et al., 1958). There were no treatment-related effects upon mortality rates, hematologies or incidences of tissue damage. These investigators also found hepatocellular enlargement and pale-staining cytoplasm in groups of three dogs exposed for 1 year to 25-1000 ppm PCNB in the diet. Two strains of mice given 1298 ppm "contaminated" PCNB in the diet for 77 weeks had increased incidences of pneumonia and lymphoid infiltration, relative to controls (BRL, 1986a). In

a more comprehensive bioassay, the NCI (1978) found that TWA concentrations of 10,064-14,635 ppm dietary PCNB led to a "hunched" appearance and abdominal urine stains in male and female rats. At these dietary levels and at lower levels (5417-7875 ppm), there were inhibitions in weight gain relative to controls. The hunched appearance also occurred in treated mice, and female mice treated with up to 9000 ppm (TWA = 8187 ppm) PCNB showed an inhibition of weight gain. No treatment-related effects or nonneoplastic tissue pathology were noted.

In the repeat study using B6C3F1 mice, there was a dose-related decreased body weight gain, increased mortality and increased incidence of ovarian abscesses in females given 2500 and 5000 ppm in the diet for 103 weeks (NTP, 1986). The increased mortality was attributed to a combination of the toxicity of PCNB and the ovarian infections. It was suggested that the PCNB-treatment predisposed the female mice to infection.

Fytzias-Danielidou (1975) administered a 75% pure suspension of PCNB to Wistar rats by gavage, 6 days/week for up to 32 weeks. Serial sacrifices were made every 8 weeks. Exposure to 400 mg/kg, and to a lesser extent 200 mg/kg led to increases in mortality, transient inhibitions in weight gain, polyuria, hepatocellular necrosis, hepatomegaly and degeneration of the renal tubular epithelium. There were no changes in hematological parameters.

A number of animal studies involving subchronic oral exposure to PCNB have been conducted. Koegel et al. (1979b) found no effects of 2 ppm dietary PCNB, given over 70-80 days, on four rhesus monkeys, relative to historic controls. In a 13-week oral dosing study, Finnegan et al. (1958) noted mortality and morbidity in rats given a dietary concentration of 5000 ppm PCNB (as a 20% dust), and inhibition in growth rate in males given 2500 ppm. At lower concentrations, there were increases in relative liver and

kidney weights. The NCI (1978) observed body weight gain depressions and mortality in rats exposed to ≥21,500 ppm PCNB, and in mice exposed to 4640 ppm (males) or 10,000 ppm (females), for a 6-week period. NTP (1986) found significantly increased relative liver weights in male mice treated at ≥2500 ppm and females at ≥5000 ppm PCNB in the diet for 13 weeks. Mice of both sexes were emaciated at ≥20,000 ppm. The 40,000 ppm level was fatal to female mice and resulted in lymphoid depletion. Finally, Kawano et al. (1979) noted evidence of increased hepatic microsomal activity in rats given 2000 ppm PCNB, of an unknown purity, for 3 weeks.

Results from the BRL (1968a) carcinogenicity bioassay are called into question, primarily because of the high percentage of HCB present as a contaminant. This compound has been found to increase incidences of hepatomas in treated hamsters and mice (Cabral et al., 1977, 1978). Findings from the NCI (1978) bioassay in mice must also be considered questionable because insufficient numbers of animals of either sex were assessed for hepatocellular tumorigenesis. The NTP (1986) repeat study in mice, however, found no evidence of carcinogenicity although the high mortality in treated females may have decreased the sensitivity of the assay.

The bioassay conducted by Van der Heijden and Til (1974) and reviewed by Albert (1977) used a sufficient number of Swiss albino mice/group and allowed for dietary administration over an extended timespan. Despite limited microscopic analysis of tissues and unknown effects of contamination with 2.7% HCB, incidence data revealed a concentration-response relationship in combined fibromas/fibrosarcomas in females. NTP (1986) and NCI (1978) did not find these tumors in B6C3F1 mice; therefore, the possibility of a difference in strain susceptibility cannot be ruled out.

A quantitative analysis of the possible influence of the technical grade contaminant HCB shows there to be a close relationship between the HCB level and the estimated carcinogenic potencies of the technical grade compound compared with HCB alone. A similar relationship seems to exist for the tumor response as well. In view of the available data, it is prudent to observe that the HCB contaminant may be influential in the observed carcinogenic activity of PCNB although the certainty of this is subject to question. At a minimum, the potency derivations to follow and criteria levels should be noted as being applicable only to technical grade PCNB, with either 11% or 2.7% contamination level, and not to PCNB alone.

Assuming a mouse consumes a daily amount of food the equivalent to 13% of its body weight (U.S. EPA, 1980), the dietary concentrations of 100, 400 and 1200 ppm correspond to 13, 52 and 156 mg/kg/day, respectively (Table 8-1). The computerized multistage model of Howe and Crump (1982) was used to compute the unadjusted q_1^* of 1.3502×10^{-3} $(mg/kg/day)^{-1}$. Multiplication of this value by the cube root of the body weight ratio of the reference human weight (70 kg) to the reference mouse weight (0.03 kg), and subsequently by the cube of the ratio of mouse lifespan (104 weeks) to duration of treatment [80 weeks (U.S. EPA, 1980)], leads to a human q_1^* of 3.93×10^{-2} $(mg/kg/day)^{-1}$. An alternative data set (Innes et al., 1969) showing hepatoma response in male and female mice with an 11% HCB contamination level gives a human q_1^* of 0.26 $(mg/kg/day)^{-1}$. Arbitrarily, this is chosen to derive criteria levels. The concentrations in water associated with risk levels of 10^{-5}, 10^{-5} and 10^{-7} are 1.33×10^{-3}, 1.33×10^{-4} and 1.33×10^{-5} mg/ℓ for a technical grade of PCNB (11% HCB contamination).

TABLE 8-1

Cancer Data Sheet for Derivation of q_1^*

Compound: PCNB (contaminated with 2.7% HCB)

Reference: Van der Heijden and Til, 1974

Species, Strain, Sex: mouse, Swiss albino, female

Body weight: 0.03 kg(assumed)

Length of exposure (le) = 80 weeks

Length of experiment (Le) = 80 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: connective tissue, fibromas/fibrosarcomas

Route, vehicle: oral, dietary

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	0/90
100	13	3/95
400	52	3/91
1200	156	12/91

Unadjusted q_1^* from study = 1.3502×10^{-3} $(mg/kg/day)^{-1}$
Human q_1^* = 3.93×10^{-2} $(mg/kg/day)^{-1}$

The U.S. EPA (1982b) listed a q_1^* of 2.62×10^{-1} $(mg/kg/day)^{-1}$ based upon hepatoma incidences in the study reported by BRL (1968a) and Innes et al. (1969) (Table 8-2). As discussed above, however, PCNB in the BRL (1968a) study was contaminated with a high level of HCB, which is known to induce hepatomas in mice (IARC, 1979).

Although the above analyses indicate that the apparent carcinogenicity of PCNB may be attributable to its contamination with HCB, they do not prove that the carcinogenicity must be attributed to HCB. The assumptions used in estimating the potency of HCB (for example, the extrapolation between species on the basis of relative body surface areas) cause these estimates, even the so-called best estimates, to be plausible upper bounds for the true potency. Hence, the numbers of tumors possibly attributable to HCB must be regarded as upper bounds and may, in fact, be lower than the number of tumors observed in the PCNB study. Therefore, the apparent carcinogenicity of PCNB cannot conclusively be explained by its contamination with HCB.

In addition, the levels of HCB in the PCNB study are much lower than the levels of HCB in the HCB study used to estimate its potency. Lower numbers of tumors attributable to HCB would be consistent with a dose-response curve for HCB that lies below a straight line. For these reasons, it can be postulated that PCNB may be carcinogenic, but other explanations, such as the HCB contamination, cannot be ruled out.

The available long-term dietary studies using relatively pure PCNB include the 2-year study in dogs (Borzelleca et al., 1971), the 80-week study in Swiss mice (Van der Heijden and Til, 1974), and the chronic bioassays in Osborne-Mendel rats (NCI, 1978) and B6C3F1 mice (NTP, 1986).

TABLE 8-2

Derivation of Human q_1^*

Compound: PCNB

Reference: Innes et al., 1969

Exposure route: oral

Species: mice

Strain: (C57B1/6 x AKR)F1

Sex: Male

Vehicle or physical state[a]: gelatin

Body weight: 0.030 kg (reported)

Duration of treatment: 539 days

Duration of study: 539 days

Lifespan of animal: 730 days (assumed)

Target organ: liver

Tumor type[b]: hepatoma

Experimental Doses or Exposures[c] (mg/kg/day)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	5/90
169	169	10/17

Human $q_1^* = 2.62 \times 10^{-1}$ $(mg/kg/day)^{-1}$

[a]Used during stomach period only (see footnote "c")

[b]Hepatoma refers to all hepatic tumors unless there were unmistakable pulmonary metastases (nonpresent)

[c]TWA dose reflecting gastric intubation (464 mg/kg/day) from days 7-28 of age (weaning), and subsequent administration in the diet (1206 ppm) for 72 weeks. TWA calculation assumed that mice consume 13% of their weight in food/day.

In the NTP (1986) study, female mice given ≥2500 ppm (≥600 mg/kg/day as determined from food consumption data) had increased incidences of ovarian abscesses resulting in dose-related increased mortality that may have been due to a PCNB-related increased susceptibility to infection. In the NCI (1978) study, rats given TWA dietary concentrations ≥5417 ppm and mice given TWA dietary concentration ≥2606 ppm had a hunched appearance that persisted throughout the later stages of the study. Assuming that rats and mice consume a daily amount of food equal to 5 and 13%, respectively, of their body weight, the TWA concentrations correspond to 271 and 339 mg/kg/day, respectively. In the Van der Heijden and Til (1974) study, mice given dietary levels of PCNB ≥100 ppm (≥13 mg/kg/day) developed dose-related increased incidences of liver hyperplastic nodules. Beagle dogs given PCNB in the diet at ≥108 ppm for 2 years developed cholestatic hepatosis with secondary bile nephrosis (Borzelleca et al., 1971). Assuming that a dog consumes a daily amount of food equal to 2.5% of its body weight, 108 ppm is equivalent to 2.7 mg/kg/day. No effects were observed at 30 ppm (0.75 mg/kg/day). Clearly, the transformed dose of 2.7 mg/kg/day represents the LOAEL for PCNB. The next lower dose at which no effects were observed is 0.75 mg/kg/day, which is, therefore, the highest NOEL.

Statements concerning available literature in this document refer to published, quotable sources and are in no way meant to imply that classified data, which this document could not address, are not in existence. These data, which are considered Confidential Business Information (CBI), are available in summary form as a CBI Appendix that can be assessed by individuals with appropriate clearance.

The data available in the CBI Appendix do not provide additional information for consideration of an alternative risk assessment, but a q_1^* of 1.37×10^{-4} $(ppm)^{-1}$ was derived by the Office of Pesticide Programs to estimate the carcinogenic risk potential of PCNB contaminated with 2.7% HCB. This derivation is for the incidence for subcutaneous fibrosarcomas alone and used nontransformed dietary levels. Furthermore, no corrections for cube root of body weight ratio or lifespan were made; therefore, the q_1^* of 1.37×10^{-4} $(ppm)^{-1}$ is the value for mice rather than for humans.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Studies on the chronic and subchronic toxicity and teratogenicity and fetoxicity of PCNB were presented in Chapter 5 and are summarized in Table 9-1. In all dietary studies, the transformed animal doses in mg/kg/day were calculated by multiplying the dietary concentration (in ppm) by 0.025 for dogs (U.S. EPA, 1985c), 0.05 for rats (U.S. EPA, 1980) or 0.13 for mice (U.S. EPA, 1980). In the oral gavage study by Fytizas-Danielidou (1975), the stated doses of 200 and 400 mg/kg/day were corrected for the limited dosing regimen (6 days/week). In the NTP (1986) study, the transformed doses were provided. The equivalent human doses were calculated by multiplying the animal transformed dose by the cube root of the ratio of the animal body weight to the reference human body weight of 70 kg.

The toxicity and teratogenicity studies by Fytizas-Danielidou (1975), Khera and Villeneuve (1975) and BRL (1968b) are not useful for RQ derivation because the samples of PCNB used were of questionable purity or were known to contain high levels of HCB, a known carcinogen (IARC, 1979) and teratogen (Courtney et al., 1976).

The subchronic studies by NCI (1978), NTP (1986) and Finnegan et al. (1958) are not considered in this RQ determination because the chronic studies examine the same or more endpoints.

As seen from Table 9-1, one of the most severe effects of PCNB exposure (excluding the studies eliminated from consideration) is the evidence of teratogenicity (clubfoot) in the offspring of mice treated with 500 mg/kg/day pure PCNB during gestation (Courtney et al., 1975). This effect occurred at an equivalent human dose of 38 mg/kg/day. Multiplying 38 mg/kg/day by 70 kg results in an MED of 2660 mg/day, which corresponds to an

TABLE 9-1

Oral Toxicity Summary for PCNB

Species/ Strain	Sex	Number at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response	Reference
Dog/beagle	M,F	4/sex/ group	12.7[a]	diet	97.8%	108 or 1080 ppm; 104 weeks	2.7 or 27	1.5 or 15	Transiently increased SGOT SGPT levels; cholestatic hepatosis and bile nephrosis	Borzelleca et al., 1971
Mouse/Swiss	M,F	100/sex/ group	0.03[a]	diet	98.2%	400 and 1200 ppm; 80 weeks	52, 156	3.9	Increased liver weights, elevated kidney weights (females)	Van der Heijden and Til, 1974
Mouse/Swiss	M,F	100/sex/ group	0.03[a]	diet	98.2%	100 ppm; 80 weeks	13	1.0	Hyperplastic liver nodules (males), neutrophilia, lymphopenia	Van der Heijden and Til, 1974
Rat/Wistar	M,F	50/sex/ group	0.35[a]	diet	98.2%	1200 ppm; 104 weeks	60	10.3	Increased liver weights; elevated kidney weights; hepatic necrosis; Mallory bodies; fatty metamorphosis	Anonymous, n.d.
Rat/Wistar	M,F	50/sex/ group	0.35[a]	diet	98.2%	400 ppm; 104 weeks	20	3.4	Hepatocellular enlargement; Mallory bodies; moderate hepatic necrosis; fatty metamorphosis	Anonymous, n.d.
Rat/Wistar	M,F	50/sex/ group	0.35[a]	diet	98.2%	100 ppm; 104 weeks	5	0.9	Hepatocellular enlargement	Anonymous, n.d.
Rat/albino	M,F	10/sex/ group	0.390 M[b] 0.253 F[b]	77% Pyrax ABB & 3% diet	technical grade	2500 ppm; 104 weeks	125	19	Inhibitions in weight gain	Finnegan et al., 1958
Rat/F344	M,F	50/group	~0.51 M[b] ~0.33 F[b]	diet	97%	5417 and 10,064 ppm (M) 7875 and 14,6351 ppm (F) 78 weeks followed by 33-35 weeks observation	271 and 503 (M), 394 and 732 (F)	53 (M) 66 (F)	Hunched appearance; abdominal staining; inhibition of weight gain	NCI, 1978
Mouse/B6C3F1	M	50/group	~0.033[b]	diet	97%	2606 and 5213 ppm, 78 weeks followed by 14-15 weeks observation	339 and 678	26	Hunched appearance	NCI, 1978

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TABLE 9-1 (cont.)

Species/ Strain	Sex	Number at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response	Reference
Rat/Wistar	M,F	20/sex/ group	~0.24[b]	suspension in methyl-cellulose	75%	100, 200 or 400 mg/kg/day, 6 days/week for 32 weeks	86, 174 or 343	13, 26 or 52	Increased mortality at ≥174 mg/kg/day; liver and kidney necrosis at ≥86 mg/kg/day	Fytizas-Danielidou, 1975
Mouse/B6C3F1	F	50/group	~0.035	diet	>99%	2500 or 5000 ppm; 103 weeks followed by 1 week observation	600 or 1400 mg/kg/day	48	Predisposition to infection; decreased body weight gain; increased mortality	NTP, 1986
Rat/albino	M,F	5/sex/ group	0.247 M[b] 0.167 F[b]	diet	technical grade	≥635 ppm; 13 weeks	≥32	4.3	Elevated liver weights; elevated kidney weights	Finnegan et al., 1958
Rat/Osborne-Mendel	M,F	5/sex	0.35[a]	diet	97%	4600 ppm; 6 weeks	2320	397	Increased mortality	NCI, 1978
Rat/Osborne Mendel	M,F	5/sex	0.35[a]	diet	97%	21,500 ppm; 6 weeks	1075	184	Body weight depression	NCI, 1978
Mouse/B6C3F1	M	5	0.03[a]	diet	97%	4640 ppm (M) 10,000 ppm (F) 6 weeks	603 (M) 1300 (F)	45	Increased mortality	NCI, 1978
Mouse/B6C3F1	M	10/group	~0.026[b]	diet	>99%	2500 (M) 20,000 (M) 13 weeks	514 6115	37 416	Increased liver weight; emaciation	NTP, 1986
Rat/Wistar	F	20/group	~0.28[b]	corn oil	NR	200 mg/kg/day days 6-15 of gestation	200	32	Decreased number of live fetuses	Khera and Villeneuve, 1975
Mouse/C57BL6	F	8-12/ group	0.03[a]	honey	contained 11% hexa-chloro-benzene	215 or 464 mg/ kg/day days 6-15 of gestation	464	35	Increased incidence of terata; renal agenesis, microphthalmia; no treatment-related maternal toxicity	BRL, 1968b
Rat/Wistar	F	NR	0.35[a]	methyl-cellulose	75%	400 mg/kg/day on days 7-10 or 12-15 of gestation	400	68	Decreased fetal body weight	Fytizas-Danielidou, 1975
Mouse/CD-1	F	10	0.03[a]	corn oil: acetone (9:1)	>99%	500 mg/kg/day gestation days 7-16	500	38	Increased fetal mortality and incidence of clubfeet	Courtney et al., 1976

[a]Reference value from U.S. EPA (1985c) for dogs and U.S. EPA (1980) for rats and mice.

[b]Estimated from body weight data provided by the investigators.

NR = Not reported

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RV_d of 1. The RV_e for teratogenicity in the absence of maternal toxicity is 10. Multiplying the RV_d by the RV_e results in a CS of 10, which corresponds to an RQ of 1000 (Table 9-2).

Of equal severity in increased mortality (RV_e=10) in mice in the NTP (1986) chronic study; however, this occurred at a higher equivalent dose and need not be scored.

The next most serious effect was liver necrosis (RV_e=6), which occurred at an equivalent dose as low as 3.4 mg/kg/day. The MED of 238 mg/kg corresponds to an RV_d of 1.9. The CS of 11.4 corresponds to an RQ of 1000. Dogs treated with an equivalent human dose of 1.5 mg/kg/day had cholestatic hepatosis and bile nephrosis (RV_e=5). The MED is 105 mg/day, which corresponds to an RV_d of 2.5. The CS of 12.5 corresponds to an RQ of 1000.

Liver hyperplasia and hypertrophy occurred at equivalent human doses of 0.9 and 1.0 mg/kg/day. Multiplying 0.9 mg/kg/day by 70 kg results in an MED of 63 mg/day, which corresponds to an RV_d of 2.8. Multiplying the RV_d by an RV_e of 3 results in a CS of 8.4, which corresponds to an RQ of 1000. As seen from Table 9-2, the highest CS resulted from the increased cholestatic hepatosis and bile nephrosis in dogs in the study by Borzelleca et al. (1971) and is recommended as the basis of the RQ (Table 9-3).

In a previous derivation for PCNB, U.S. EPA (1983a) derived a RQ of 1000 based on an abstract of the study by Fytizas-Danielidou (1975). As discussed above, the purity of the PCNB used in the study was only 75%. The current determination evaluated more appropriate data.

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Evidence for the potential carcinogenicity of PCNB was presented in Section 5.1. PCNB of unspecified purity was positive as a tumor initiator

TABLE 9-2

Oral Composite Scores for PCNB

Species	Animal Dose (mg/kg/day)	Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
Rat	5	63	2.8	Enlargement of hepatocytes; Mallory bodies; fine vacuolar metamorphosis of hepatocytes	3	8.4	1000	Anonymous, n.d.
Rat	20	238	1.9	Enlarged hepatocytes with Mallory bodies; fatty metamorphosis; moderate centrilobular cell necrosis	6	11.4	1000	Anonymous, n.d.
Mouse	500	2660	1.0	Elevated fetal mortality; increased incidence of clubfoot	10	10	1000	Courtney et al., 1976
Dog	2.7	105	2.5	Cholestatic hepatosis and bile nephrosis	5	12.5	1000	Borzelleca et al., 1971

TABLE 9-3

PCNB

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose:	105 mg/day
Effect:	cholestatic hepatosis and bile nephrosis
Reference:	Borzelleca et al., 1971
RV_d:	2.5
RV_e:	5
Composite Score:	12.5
RQ:	1000

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*Equivalent human dose

when applied to the skin of mice followed by promotion with croton oil (Searle, 1966). Several studies, which indicated that PCNB may exert a weak carcinogenic effect, were subsequently found to require additional interpretation from a risk assessment perspective. BRL (1968a) and Innes et al. (1969) treated two strains of mice with 1298 ppm dietary PCNB for 77 weeks; the incidence of hepatoma was elevated. Courtney et al. (1976) later revealed, however, that the PCNB sample was contaminated with 11% HCB, a known hepatic carcinogen in hamsters and mice (IARC, 1979). The NCI (1978) found a slightly increased incidence of hepatocellular carcinomas in B6C3F1, given lifetime exposure to a TWA concentration of 8187 ppm PCNB. The small number of mice analyzed histologically and the variable dietary concentration manipulations significantly detracted from the significance of this finding, however.

A repeat study in B6C3F1 mice by NTP (1986) failed to find any evidence of carcinogenicity in males or females treated with highly pure PCNB (>99% PCNB, 0.07±0.01% HCB) at dietary levels of 2500 or 5000 ppm for 103 weeks.

In a previous determination (U.S. EPA, 1983b), the study by BRL (1968a) was used as the basis for an F factor of 1.4 $(mg/kg/day)^{-1}$, placing PCNB in Potency Group 2. The evidence was considered to be limited in animals and inadequate in humans, placing PCNB in IARC Group 3. The hazard ranking was low. As discussed above, the BRL (1968a) used a grade of PCNB that contained a high level (~11%) of HCB. An alternative analysis uses an unpublished study by Van der Heijden and Til (1974), which used PCNB with an HCB content of 2.7%.

As discussed in Section 7.1., the U.S. EPA (1982b) concluded that "the evidence does not positively establish a correlation between exposure to PCNB itself and oncogenicity" but that HCB was probably the carcinogenic

agent. In either case, it is only appropriate to derive an F factor for PCNB contaminated with HCB and not assign a value to PCNB alone.

In the study by Van der Heijden and Til (1974), 80-week dietary exposure of female Swiss mice to 100, 400 or 1200 ppm PCNB (containing 2.7% HCB) led to a concentration-related increase in the incidences of fibrosarcomas and total fibromas/fibrosarcomas (see Table 5-3). Assuming that a mouse consumes the equivalent of 13% of its body weight daily, these concentrations correspond to 13, 52 and 156 mg/kg/day. When applied to the Howe and Crump (1982) computerized multistage model, the maximum likelihood estimate of the dose producing a carcinogenic response in 10% of the population is 117.667, for a $1/ED_{10}$ of 8.4986×10^{-3} (Table 9-4). After adjustment for both the cube root ratio of human to mouse body weight (0.70 kg:0.03 kg) and the cube of the ratio of the lifespan of the mouse to the duration of treatment $(104:80)^3$, a human F-factor of 2.4764×10^{-1} $(mg/kg/day)^{-1}$ is obtained. As previously mentioned, the human F-factor varies from 2.5×10^{-1} $(mg/kg/day)^{-1}$ to 1.4, depending upon which study (based on level of HCB contamination, Table 9-5) is used. Given the uncertainty, the higher value of 1.4 is selected for RQ purposes, noting that this applies to a technical grade of PCNB with 11% HCB contamination. In the absence of human data, and the existence of primarily negative mutagenicity data and the limited evidence from animal studies, PCNB and technical grade PCNB should be considered a CAG Group C chemical (U.S. EPA, 1984). Group C chemicals with an F <100 are rated LOW on the CERCLA Hazard Ranking Scheme.

TABLE 9-4

Derivation of Potency Factor (F)
for PCNB (contaminated with 2.7% HCB)

Reference:	Van der Heijden and Til (1974)			
Exposure route:	oral			
Species:	mouse			
Strain:	SPF/Swiss			
Sex:	F			
Vehicle or physical state:	diet			
Body weight:	0.03 kg			
Duration of treatment:	80 weeks			
Duration of study:	80 weeks			
Lifespan of animal:	104 weeks			
Target organ:	subcutaneous connective tissue			
Tumor type:	combined fibromas/fibrosarcomas			
Experimental doses/exposure (ppm):	0	100	400	1200
Transformed doses (mg/kg/day):	0	13	52	156
Tumor incidence:	0/90	3/95	3/91	12/91
Unadjusted $1/ED_{10}$:	8.4986×10^{-3} (mg/kg/day)$^{-1}$			
Adjusted $1/ED_{10}$ (F factor):	2.48×10^{-1} (mg/kg/day)$^{-1}$			

TABLE 9-5

PCNB Potency Attributable to HCB Contamination

ANALYSIS 1. Relative Potencies of PCNB and HCB

PCNB+HCB potency (q_1*)	0.0393
HCB potency (q_1*)	1.6700
Ratio	2.4%
Actual contamination level	2.7%

CONCLUSION: PCNB+HCB potency is no more than what would be expected from HCB contamination alone.

ANALYSIS 2. Tumors Attributable to HCB

PCNB+HCB doses (ppm diet)	100	400	1200
(mg/kg/day)	13	52	156
HCB doses (2.7% of PCNB+HCB doses)	0.351	1.404	4.212
HCB potency (human q_1*)	1.67	1.67	1.67
(mouse q_1*)	0.126	0.126	0.126
Mice tested for PCNB+HCB	95	91	91
Mice with tumors	3	3	12
Max attributable to HCB (linear)	4.2	16.1	48.3
(1-stage)	4.1	14.7	37.5

CONCLUSION: Tumors may be attributable to HCB contamination.

10. REFERENCES

Albert, R.E. 1977. The carcinogen assessment group's preliminary report on pentachloronitrobenzene (PCNB). U.S. EPA, CAG, Washington, DC.

Alexander, M. 1981. Biodegradation of chemicals of environmental concern. Science. 211: 132-138.

Alkinson, R. 1985. Kinetics and mechanisms of the gas phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions. Submitted to Chem. Rev.

Ando, M., S. Hirano and Y. Itoh. 1985. Transfer of hexachlorobenzene (HCB) from mother to newborn baby through placenta and milk. Arch. Toxicol. 56: 3. p. 195-200.

Anonymous. n.d. Carcinogenicity study with pentachloronitrobenzene (PCNB) in rats. Report No. A02007, Central Institute for Food and Nutrition, Netherlands. (Confidential) (Cited in Albert, 1977; U.S. EPA, 1977)

Bahig, M.E., A. Kraus, W. Klein and F. Korte. 1981. Metabolism of pentachloronitrobenzene-14C (quintozene) in fish. Chemosphere. 10(3): 319-322.

Beck, J. and K.E. Hansen. 1974. The degradation of quintozene, pentachlorobenzene, hexachlorobenzene, and pentachloroaniline in soil. Pestic. Sci. 5: 41-48.

Betts, J.J., S.P. James and W.V. Thorpe. 1955. Metabolism of pentachloronitrobenzene and 2,3,4,6-tetrachloronitrobenzene and the formation of mercapture acids in the rabbit. Biochem. J. 61: 611-617.

Borzelleca, J.F. and P.S. Larson. 1968. Toxicity study of the effect of adding Terraclor to the diet of beagle dogs for a period of two years. Unpublished report prepared by the Dept. of Pharmacology, Medical College of Virginia. Submitted by Olin Corp. as Report No. 2490. EPA Acc. No. 248283, June 10.

Borzelleca, J.R., P.S. Larson, E.M. Crawford, G.R. Hennigar and E.J. Kuchar. 1971. Toxicologic and metabolic studies on pentachloronitrobenzene. Toxicol. Appl. Pharmacol. 18: 522-534.

Bourquin, A.W. 1973. Estuarine microbes and organochlorine pesticides (a brief review). In: The Microbial Degradation of Oil Pollutants. Center for Wetland Resources, Louisiana State University, Baton Rouge, LA. p. 237-243. LSU-SG-73-01.

BRL (Bionetics Research Laboratories). 1968a. Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. Vol. 1. Carcinogenic Study. Prepared by Bionetics Research Labs, Inc., Bethesda, MD, for NCI. NTIS PB-223159.

BRL (Bionetics Research Laboratories). 1968b. Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. Volume II. Teratogenic study in mice and rats. Prepared by Bionetics Research Labs., Inc., Bethesda, MD, for NCI. NTIS PB-223 160/3.

Buselmaier, W., G. Roehrborn and P. Propping. 1972. Mutagenicity investigations with pesticides in the host-mediated assay and the dominant lethal test in mice. Biol. Zentralbl. 91: 311-325.

Buser, H.R. and H.P. Bosshardt. 1975. Studies on the possible formation of polychloroazobenzenes in quintozene treated soil. Pest. Sci. 6: 35-41.

Cabral, J.R.P., P. Shubik, T. Mollner and F. Raitamo. 1977. Carcinogenic activity of hexachlorobenzene in hamsters. Nature. 269: 510-511.

Cabral, J.R.P., T. Mollner, F. Raitano and P. Shubik. 1978. Carcinogenesis study in mice with hexachlorobenzene (Abstract No. 242). Toxicol. Appl. Pharmacol. 45: 323.

Cairns, T., E.G. Siegmund and H.T. Masumoto. 1983. Occurrence of pentachloronitrobenzene and its metabolites in spinach leaves. Bull. Environ. Contam. Toxicol. 31: 230-234.

Casley, J.C. 1968. The loss of three chloronitrobenzene fungicides from the soil. Bull. Environ. Contam. Toxicol. 3: 180-193.

Chacko, C.I., J.L. Lockwood and M. Zabik. 1966. Chlorinated hydrocarbon pesticides: Degradation by microbes. Science. 154: 893-895.

Cholakis, J.M., M.J. McKee, L.C. Wong and J.D. Gile. 1981. Acute and subacute toxicity of pesticides in microtine rodents. ASTM Spec. Tech. Publ. 757: 143-154.

Clarke, C.H. 1971. The mutagenic specificities of pentachloronitrobenzene and captan, two environmental mutagens. Mutat. Res. 11: 247-248.

Cole, L.K. and R.L. Metcalf. 1977. Distribution of pesticides and their derivatives in the air, soil, water and biota of physical model ecosystems. Proc. Annu. Meet. Air Pollut. Control Assoc. 77-45.5: 15.

Courtney, K.D., M.F. Copeland and A. Robbins. 1976. The effects of pentachloronitrobenzene and related compounds on fetal development. Toxicol. Appl. Pharmacol. 35(2): 239-256.

Crosby, D.G. and N. Hamadmad. 1971. The photoreduction of pentachlorobenzenes. J. Agric. Food Chem. 19: 1171-1174.

Dejonckheere, W., J. Willocx, W. Steurbaut, R.H. Kips, J.P. Voets and W. Verstraete. 1975a. Changes in the rate of metabolism of quintozene in the soil under varying microbial growth conditions. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 40: 1187-1197.

Dejonckheere, W., W. Steurbaut and R.H. Kips. 1975b. Residues of quintozene, hexachlorobenzene, dichloran, and pentachloroaniline in soil and lettuce. Bull. Environ. Contam. Toxicol. 13: 720-729.

Devos, R.H., M.C. Tennoever De Braun and P.D.A. Olthof. 1974. Residues of pentachloronitrobenzene and related compounds in greenhouse soils. Bull. Environ. Contam. Toxicol. 11: 567-571.

Dive, D., H. Leclerc and G. Persoone. 1980. Pesticide toxicity on the ciliate protozoan Colpidium campylum: Possible consequences of the effect of pesticides in the aquatic environment. Ecotoxicol. Environ. Saf. 4(2): 129-133.

Duggan, R.E., H.C. Barry and L.Y. Johnson. 1966. Pesticide residues in total diet samples. Science. 151: 101-104.

Feuersenger, M. 1961. Residue determinations of cabbage after treatment with KP2 (Pentachloronitrobenzene). Mutt. Biol. Bundesanstalt Land-Forstwirtsch. 104: 155-157.

Finnegan, J.K., P.S. Larson, S.R. Blackwell, H.B. Haag and G.R. Hennigar. 1958. Acute and chronic toxicity studies on pentachloronitrobenzene. Arch. Int. Pharm. p. 38-52.

Freitag, D., H. Geyer, W. Klein, A.G. Kraus, E. Lahaniatis and F. Korte. 1979. An approach for comparative screening of the environmental behavior of chemical. Ecotoxicol. Environ. Saf. 3: 144-151.

Freitag, D., H. Geyer, A. Kraus, R. Viswanathan and D. Kotzias. 1982. Ecotoxicological profile analysis. VII. Screening chemicals for their environmental behavior by comparative evaluation. Ecotoxicol. Environ. Saf. p. 60-81.

Fugita, K., H. Suzuki and F. Ochiai. 1976. Keratoconjunctivites due to pesticides of relatively low acute toxicity. (Abstract) (Jap.) Rinsho Ganka. 30(4): 419-423.

Fytizas-Danielidou, R. 1975. Some aspects of the chronic toxicity of quintozene (pentachloronitrobenzene) in rats. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 40: 1175-1185.

Gile, J.D. and J.W. Gillett. 1979. Fate of selected fungicides in a terrestrial laboratory ecosystem. J. Agric. Food Chem. 27: 115. p. 9-64.

Hansch, C. and A.J. Leo. 1985. MedChem Project. Issue no. 26. Pomona College, Claremont, CA.

Hartley, D. and H. Kidd. 1983. The Agrochemicals Handbook. The Royal Society of Chemistry, Nottingham, England. p. A361.

Hasimoto, Y. and Y. Nishiuchi. 1981. Establishment of bioassay methods for the evaluation of acute toxicity of pesticides to aquatic organisms. J. Pest. Sci. 6(2): 257-264.

Hawley, G.G. 1981. The Condensed Chemical Dictionary, 10th ed. Van Nostrand Reinhold Co., New York. p. 782.

Haworth, S., T. Lawlor, K. Mortelmans, W. Speck and E. Zeiger. 1983. Salmonella mutagenicity test results for 250 chemicals. Em. Mutagenesis Suppl. p. 3-142.

Helling, C.S., D.G. Dennison and D.D. Kaufman. 1974. Fungicide movement in soils. Phytopathology. 64: 1091-1100.

Howe, R.B. and K.S. Crump. 1982. GLOBAL 82. A computer program to extrapolate quantal animal toxicity data to low doses. Office of Carcinogen Standards, OSHA, U.S. Dept. Labor. 41USC252c3.

IARC (International Agency for Research on Cancer). 1979. Hexachlorobenzene. In: Some Halogenated Hydrocarbons. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyon, France. Vol. 20, p. 155-178.

Innes, J.R.M., B.M. Ulland, M.G. Valeria, et al. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. 41: 1101-1114.

Isensee, A.R. and R.S. Yockim. 1980. Freshwater micro-ecosystem development and testing of substitute chemicals. EPA-600/3-80-008. NTIS PB80-151533. 39 p.

Johnson, R.D. and D.D. Manske. 1976. Pesticide residues in total diet samples (IX). Pestic. Monit. J. 9: 157-169.

Johnson, R.D. and D.D. Manske. 1977. Pesticide residues in total diet samples (IX). Pestic. Monit. J. 11: 116-131.

Johnson, R.D., D.D. Manske and D.S. Podrebac. 1981a. Pesticide, heavy metal and other chemical residues in adult total diet samples. Pestic. Monit. 15: 54-69.

Johnson, R.D., D.D. Manske, D.H. New and D.S. Podrebac. 1981b. Pesticide, heavy metal, and other chemical residues in infant and toddler total diet samples. Pestic. Monit. 15: 39-50.

Johnson, R.D., D.D. Manske and D.S. Podrebarac. 1984a. Pesticide, heavy metal, and other chemical residues in adult total diet samples. J. Assoc. Off. Anal. Chem. 67: 154-166.

Johnson, R.D., D.D. Manske, D.H. New and D.S. Podrebarac. 1984b. Pesticide, heavy metal, and other chemical residues in infant and toddler total diet samples. J. Assoc. Off. Anal. Chem. 67(1): 145-154.

Jordan, R.L., F. Sperling, H. Klein and J.F. Borzelleca. 1975. Study of the potential teratogenic effects of pentachloronitrobenzene in rats. Toxicol. Appl. Pharmacol. 33: 222-230.

Jorgenson, T.A., C.J. Rushbrook and G.W. Newell. 1976. In vivo mutagenesis investigations of ten commercial pesticides. Toxicol. Appl. Pharmacol. 37(1): 109.

Kanazawa, J. 1981. Measurement of the biocentration factors of pesticides by freshwater fish and their correlation with physicochemical properties or acute toxicities. Pestic. Sci. 12(4): 417-424.

Kawano, S., K. Hirose, S. Iguchi and K. Hiraga. 1979. Toxicological studies of polychlorinated aromatic compounds in female rats. Tokyo-toritsu Eisei Kenkyusho Kenkyu Nempo. 30-2: 116-123.

Khera, K.S. and D.C. Villeneuve. 1975. Teratogenciity studies on halogenated benzenes (pentachloro-, pentachloronitro- and hexabromo-) in rats. Toxicology. 5: 117-122.

Ko, W.H. and J.D. Farley. 1969. Conversion of pentachloronitrobenzene to pentachloroaniline in soil and the effect of these compounds on soil microorganisms. Phytopathology. 59: 64-67.

Koegel, W., W.F. Mueller, F. Coulston and F. Korte. 1979a. Fate and effects of pentachloronitrobenzene in rhesus monkeys. J. Agric. Food Chem. 27(6): 1181-1185.

Koegel, W., W.F. Mueller, F. Coulston and F. Korte. 1979b. Uptake, body distribution, storage, and excretion of pentachloronitrobenzene-^{14}C in rhesus monkeys. Chemosphere. 8(2): 89-95.

Koegel, W., W.F. Mueller, F. Coulston and F. Korte. 1979c. Biotransformation of pentachloronitrobenzene-^{14}C in rhesus monkeys after single and chronic oral administration. Chemosphere. 8(2): 96-105.

Komala, Z. 1976. Further investigation on the effect of pesticides on Paramecium aurelia. Folia Biol. (Krakow). 24(1): 65-76.

Korte, F. and W. Klein. 1982. Degradation of benzene in the environment. Ecotoxicol. Environ. Saf. 6: 311-327.

Korte, F., D. Freitag, H. Geyer, W. Klein, A.G. Kraus and E. Lahaniatis. 1978. Ecotoxicologic profile analysis. A concept for establishing ecotoxicologic priority lists for chemicals. Chemosphere. 7(1): 79-102.

Kotzias, D., W. Klein, F. Lotz, S. Nitz and F. Korte. 1979. The photo-induced mineralization of organic chemicals. Chemosphere. 5: 301-304.

Kuchar, E.J., F.O. Geenty, W.P. Griffith and R.J. Raymond. 1969. Analytical studies of metabolism of Terraclor in beagle dogs, rats and plants. J. Agric. Food Chem. 17(6): 1237-1240.

Kuroda, K. 1975. Lethal effect of pesticides on saghalien trout fry. Mizu Shori Gijutsu (Water Purification Liquid Wastes Treatment). 16(5): 441-448.

Lyman, W.J., W.F. Reehl and D.H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods Environmental Behavior of Organic Compounds. McGray-Hill Book Co., New York. p. 15-16, 4-9.

Manske, D.D. and R.D. Johnson. 1975. Pesticide residues in total diet samples (VIII). Pestic. Monit. 9: 94-105.

Manske, D.D. and R.D. Johnson. 1977. Pesticide and other chemical residues in total diet samples. Pestic. Monit. 10: 134-148.

McLeod, H.A., D.C. Smith and N. Bluman. 1980. Pesticide residues in the total diet in Canada. J. Food Saf. 2: 141-164.

Metcalf, R.L., L.K. Cole, S.G. Wood, D.J. Mandel and M.L. Milbrath. 1979. Design and evaluation of a terrestrial model ecosystem for evaluation of substitute pesticide chemicals. EPA/600/3-79/004. p. 308.

Mohn, G. 1971. Microorganisms as test systems for mutagenicity. Arch. Toxikol. 28(2): 93-104.

Moriya, M., T. Ohta, K. Watanabe, T. Miyazawa, K. Kato and Y. Shirasu. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. 116(3-4): 185-186.

Murphy, S.E., A. Drotar and R. Fall. 1982. Biotransformation of the fungicide, pentachloronitrobenzene, by Tetrahymena thermophila. Chemosphere. 11(1): 33-40.

Murthy, N.B.K. and D.D. Kaufman. 1978. Degradation of pentachloronitrobenzene (PCNB) in anaerobic soils. J. Agric. Food Chem. 26: 1151-1156.

NCI (National Cancer Institute). 1978. Bioassay of pentachloronitrobenzene for possible carcinogenicity. DHEW/PUB/NIH-78-1311. p. 1311.

NTP (National Toxicology Program). 1986. Toxicology and Carcinogenesis Studies of Pentachloronitrobenzene (CAS No. 82-68-8) in B6C3F1 Mice (Feed Studies). Galley Draft Prepared April 1986. NTP-TR-325. U.S. DHHS, PHS, NIH.

O'Grodnick, J.S., J.A. Adamovics, S.H. Blake and J. Wedig. 1981. The metabolic fate of 14C-labeled pentachloronitrobenzene in Osborne-Mendel rats. Chemosphere. 10(1): 67-72.

Oliver, B.G. and A.J. Niimi. 1985. Bioconcentration factors of some halogenated organics for rainbow trout: Limitations in their use for prediction of environmental residues. Environ. Sci. Technol. 19(9): 842-849.

Podrebarac, D.S. 1984a. Pesticide, heavy metal, and other chemical residues in adult total diet samples. J. Assoc. Off. Anal. Chem. 67: 176-185.

Podrebarac, D.S. 1984b. Pesticide, heavy metal, and other chemicals residues in infant and toddler total diet samples. J. Assoc. Off. Anal. Chem. 67(1): 166-175.

Poels, C.L., M.A. van der Gaag and J.F.J. van do Kerkhoff. 1980. An investigation into the long-term effects of Rhine water on rainbow trout. Water Res. 14(8): 1029-1035.

Rautapaa, J., H. Pyysalo and H. Blomavist. 1977. Quintozene in some soils and plants in Finland. Ann. Agric. Fenn. 16: 277-282.

Renner, G. 1980. Toxicities of combinations of pentachloronitrobenzene with mercuric chloride or cadmium chloride, and hexachlorobenzene with mercuric chloride administered to rats. Xenobiotica. 10(7-8): 551-556.

Renner, G. 1981. Biotransformation of the fungicides hexachlorobenzene and pentachloronitrobenzene. Xenobiotica. 11(7): 435-446.

Renner, G. and P.T. Nguyen. 1984. Mechanisms of the reductive denitration of pentachloronitrobenzene (PCNB) and the reductive dechlorination of hexachlorobenzene (HCB). Xenobiotica. 14(9): 705-710.

Schauerte, W., J.P. Lay, W. Klein and F. Korte. 1982. Long-term fate of organochlorine xenobiotics in aquatic ecosystems. Ectotox. Environ. Saf. 6: 560-569.

Schumann, A.M. and J.F. Borzelleca. 1978. An assessment of the methemoglobin and Heinz body-inducing capacity of pentachloronitrobenzene in the cat. Toxicol. Appl. Pharmacol. 44(3): 523-529.

Searle, C.E. 1966. Tumor initiatory activity of some chloromononitrobenzenes and other compounds. Cancer Res. 26: 12-17.

Shirasu, Y., M. Moriya, K. Kato, A. Furuhashi and T. Kada. 1976. Mutagenicity screening of pesticides in the microbial system. Mutat. Res. 40: 19-30.

SRI (Stanford Research Institute). 1984. 1984 Directory of Chemical Producers, United States of America. SRI International, Menlo Park, CA. p. 774.

SRI (Stanford Research Institute). 1985. 1985 Directory of Chemical Producers, United States of America. SRI International, Menlo Park, CA.

U.S. EPA. 1977. Rebuttable presumption against registration and continued registration of pesticide products containing pentachloronitrobenzene (PCNB). Federal Register. 42(203): 56072-562100.

U.S. EPA. 1980. Guidelines and methodology used in the preparation of health effects assessment chapters of the consent decree water criteria documents. Federal Register. 45(231): 79347-73357.

U.S. EPA. 1982a. Health effects assessment summary for 300 hazardous organic constituents in support of regulatory impact analysis of the land disposal branch (LDB) and interim final incinerator regulations of the Technical Branch (TB) of the Office of Solid Wastes. U.S. EPA, ECAO, Cincinnati, OH.

U.S. EPA. 1982b. Pentachloronitrobenzene (PCNB); notice of determination concluding the rebuttable presumption against registration. Federal Register. 47(82): 18177-18179.

U.S. EPA. 1982c. Code of Federal Regulations. 40: 180.291.

U.S. EPA. 1982d. Health Effects Assessment of Pentachloronitrobenzene. Prepared by Syracuse Research Corporation, Syracuse, NY, under Contract No. 68-03-3112. ECAO, U.S. EPA, Cincinnati, OH.

U.S. EPA. 1983a. Reportable quantity document for benzene, pentachloronitro. ECAO, U.S. EPA, Cincinnati, OH.

U.S. EPA. 1983b. Technical Support Document and Summary Table for the Ranking of hazardous Chemicals Based on Carcinogenicity. U.S. EPA, Washington, DC. OHEA-C-073.

U.S. EPA. 1984. Guidelines for carcinogenic risk assessment. Federal Register. 49(227): 46294-46301. CAG, U.S. EPA, Washington, DC.

U.S. EPA. 1985a. OHMTADS (Oil and Hazardous Material Technical Assistance Data System). On-line.

U.S. EPA. 1985b. Summary of Acceptable Daily Intakes (ADIs) for Oral Exposure. March, 1985. ECAO, U.S. EPA, Cincinnati, Ohio.

U.S. EPA. 1985c. Reference Values for Risk Assessment. ECAO, Cincinnati, OH. ECAO-CIN-477.

U.S. EPA. 1986. Reference doses (RfDs) for oral exposure. Pentachloronitrobenzene (PCNB). U.S. EPA, OPP, Washington, DC.

USITC (U.S. International Trade Commission). 1985. Synthetic Organic Chemicals. U.S. Production and Sales, 1984. USITC Publ. 1745, Washington, DC.

Valange, B. 1974. Pesticide residues in fruits and vegetables. Results of the investigation performed in 1972. Parasitica. 30(1): 13-21.

Valange, B. and J. Henriet. 1974. Organochlorine pesticide residues in corn and dried sugar beet pulp. Rev. Agric. (Brussels). 27(4): 871-879.

Van der Heijden, C.A. and M.P. Til. 1974. Pentechloronitrobenzene (PCNB) carcinogenicity study in mice. Report No. R4365. Central Institute for Food and Nutrition, The Netherlands. (Confidential) (Cited in U.S. EPA, 1977)

Villeneuve, D.C. and K.S. Khera. 1975. Placental transfer of halogenated benzenes (Pentachloro-, Pentachloronitro-, and Hexabromo-) in rats. Environ. Physiol. Biochem. 5: 328-331.

Vogel, E. and J. Chandler. 1974. Mutagencity testing of cyclamate and some pesticides in Drosophila melanogaster. Experimentia. 30: 621-623.

Wang, C.H. and F.E. Broadbent. 1972. Kinetics of lesses of PCNB and DCNA in three California soils. Soil Sci. Soc. Am. Proc. 36: 74-2-5.

Waters, M.D., S.S. Sandhu, V.F. Simmon, et al. 1982. Study of pesticide genotoxicity. Basic Life Sci. 21: 275-326.

WHO/FAO (World Health Organization/Food and Agriculture Organization). 1974. Pesticide residues in food report of the 1973 joint meeting of the FAO working party of experts on pesticide residues and the WHO expert committee on pesticide residues Geneva, Switzerland. November 26-December 5, 1973. WHO Tech. Rep. Ser. 545: 7-42.

Worthing, C.R. 1983. The Pesticide Manual, 76th ed. The British Crop Protection Council, Croydon, England. p. 484.

Yeboah, P.O. and W.W. Kilgore. 1984. Analysis of airborne pesticides in a commercial pesticide storage building. Bull. Environ. Contam. Toxicol. 32: 629-634.

APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in October, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1985. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1985-1986. Cincinnati, OH. 114 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., Littleton, MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyons, France.

ITII (International Technical Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1986. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, N.I. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1984. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1985. Status Report on Rebuttable Presumption Against Registration (RPAR) or Special Review Process. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1985. CSB Existing Chemical Assessment Tracking System. Name and CAS Number Ordered Indexes. Office of Toxic Substances, Washington, DC.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. U.S. Production and Sales, 1982, USITC Publ. 1422, Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. Office of Pesticide Programs, U.S. EPA, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.